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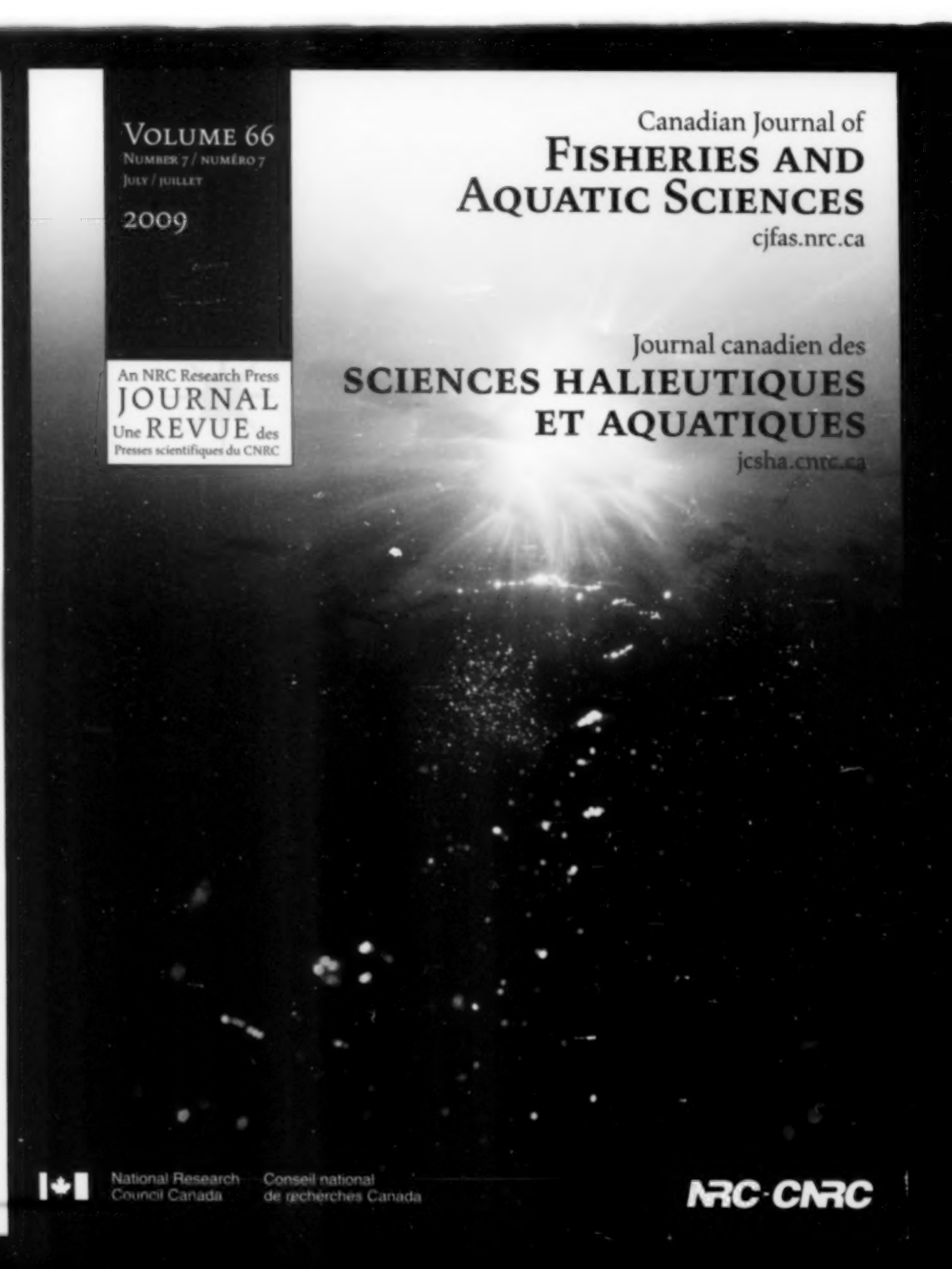

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ANNOUNCEMENT / COMMUNIQUÉ

A new Co-Editor for CJFAS

I am pleased to announce the appointment of Dr. Rolf Vinebrooke as Co-Editor of the *Canadian Journal of Fisheries and Aquatic Sciences*. Dr. Vinebrooke earned his B.A. at the University of Winnipeg, his B.Sc. and M.Sc. at the University of Toronto, and his Ph.D. at the University of Regina. He is Associate Professor and Associate Chair of Graduate Studies in the Department of Biological Sciences at the University of Alberta. His research focuses on the cumulative effects of multiple human stressors on arctic, boreal, mountain, and prairie aquatic ecosystems. Rolf has been involved with the *Canadian Journal of Fisheries and Aquatic Sciences* as an Associate Editor since 2003 and as a Vice-Editor since 2007. He joins Don Jackson as Co-Editor of the Journal.

Welcome aboard Rolf!

Bruce Dancik
Editor-in-Chief
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Nouveau corédacteur en chef au JCSHA

Je suis heureux d'annoncer la nomination de M. Rolf Vinebrooke au poste de corédacteur en chef du *Journal canadien des sciences halieutiques et aquatiques*. M. Vinebrooke est titulaire d'un B.A. de l'Université de Winnipeg, d'un B.Sc. et d'un M.Sc. de l'Université de Toronto, et d'un Ph.D. de l'Université de Regina. Il est professeur agrégé et président associé des études supérieures au Département des sciences biologiques de l'Université de l'Alberta. Ses travaux de recherche portent sur les effets cumulatifs des perturbations anthropiques sur les écosystèmes aquatiques en régions arctiques, boréales et montagneuses et dans les prairies. Rolf est membre du Comité de rédaction du *Journal canadien des sciences halieutiques et aquatiques* depuis 2003 et sous-rédacteur en chef depuis 2007. Il se joint à Don Jackson comme corédacteur en chef de la revue.

Bienvenue à bord, Rolf!

Bruce Dancik
Directeur de la publication
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ANNOUNCEMENT / COMMUNIQUÉ

A new Co-Editor for CJFAS

It gives me great pleasure to have Dr. Rolf Vinebrooke join me as Co-Editor of the *Canadian Journal of Fisheries and Aquatic Sciences*. Rolf has been actively involved with the Journal for a number of years and has served as Vice-Editor during the past two years. In that capacity, he handled manuscripts in the standard Associate Editor mode, but also oversaw many manuscripts as "Editor" where I was in conflict of interest. Knowing how well Rolf has handled these responsibilities and his scientific excellence, I am pleased that he agreed to become more actively involved in helping set the Journal's direction. Rolf brings a strong background in the aquatic sciences ranging across many trophic levels, ecosystems, and types of ecological questions. I thank Rolf for his willingness to take on the added responsibilities, and I look forward to working with him in this new capacity.

Don Jackson
Editor
Canadian Journal of Fisheries and Aquatic Sciences

Nouveau corédacteur en chef au JCSHA

C'est avec un très grand plaisir que j'accueille M. Rolf Vinebrooke au poste de corédacteur en chef du *Journal canadien des sciences halieutiques et aquatiques*. Rolf collabore déjà activement à la revue depuis bon nombre d'années. Il a servi à titre de sous-rédacteur en chef au cours des deux dernières années, où il traitait les manuscrits comme « membre du Comité de rédaction » et comme « rédacteur en chef » lorsque j'étais en position de conflit d'intérêts. Connaissant sa rigueur scientifique et la façon dont il a assumé avec brio ces responsabilités, je suis heureux qu'il ait accepté de s'impliquer davantage dans l'orientation de la revue. Rolf a de solides antécédents dans le domaine des sciences aquatiques, qu'il s'agisse de niveaux trophiques, d'écosystèmes ou de problèmes écologiques variés. Je tiens à le remercier d'avoir accepté de nouvelles responsabilités et j'ai bien hâte de collaborer avec lui.

Don Jackson
Rédacteur en chef
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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Experimental measurement of hydrosystem-induced delayed mortality in juvenile Snake River spring Chinook salmon (*Oncorhynchus tshawytscha*) using a large-scale acoustic array

Erin L. Rechisky, David W. Welch, Aswea D. Porter, Melinda C. Jacobs, and Adrian Ladouceur

Abstract: Out-migrating Snake River salmon smolts must pass eight major hydro dams before reaching the Pacific Ocean. Direct mortality at the dams is generally low; however, the cumulative stress caused by dam passage is hypothesized to result in delayed mortality, which occurs beyond the impounded section of the river. We tested the delayed mortality hypothesis by comparing in-river and early ocean survival of hatchery-origin spring Chinook salmon (*Oncorhynchus tshawytscha*) from the Snake River to a mid-Columbia River population that passes through only four dams and has higher smolt to adult return rates. Smolts >140 mm fork length were implanted with acoustic transmitters and tracked with the Pacific Ocean Shelf Tracking (POST) array to as far as Alaska. There was no detectable difference in survivorship to the first ocean detection line, 274 km beyond the final dam (S_{Snake} = 29% ± 4%, S_{Yakima} = 28% ± 5%), indicating that the survival disparity observed in adult return rates may develop later in the marine life history phase. Our study is the first to estimate survival in the coastal ocean and demonstrates the utility of a large-scale array in testing previously intractable hypotheses.

Résumé : Les saumoneaux qui émigrent de la rivière Snake doivent traverser huit barrages hydroélectriques importants avant de rejoindre le Pacifique. La mortalité directe aux barrages est généralement basse; cependant, nous avançons l'hypothèse selon laquelle le stress cumulatif dû au passage des barrages cause une mortalité différée qui se produit au-delà la section de la rivière pourvue de barrages. Nous avons testé l'hypothèse de la mortalité différée en comparant la survie dans la rivière et au début de la période océanique chez des saumons chinook (*Oncorhynchus tshawytscha*) printaniers de pisciculture avec celle d'une population de la région médiane du Columbia qui ne traverse que quatre barrages et qui possède un taux de retour de saumoneaux à adultes plus élevé. Nous avons implanté des émetteurs acoustiques à des saumoneaux de longueur à la fourche >140 mm et avons suivi les poissons par l'intermédiaire du réseau POST (surveillance de la plate-forme du Pacifique) parfois jusqu'en Alaska. Il n'y a pas de différence décelable de survie au niveau de la première ligne océanique de détection à 274 km au-delà du dernier barrage (S_{Snake} = 29% ± 4%, S_{Yakima} = 28% ± 5%), ce qui indique que la différence de survie observée dans les taux de retour des adultes peut se développer plus tard durant la phase marine du cycle biologique. Notre étude est la première à estimer la survie dans l'océan côtier et elle démontre l'utilité d'un réseau à grande échelle pour tester des hypothèses jusqu'à maintenant irréductibles.

[Traduit par la Rédaction]

Introduction

Out-migrating Snake River Chinook salmon (*Oncorhynchus tshawytscha*) smolts must pass four major hydro dams in the Snake River and four in the lower Columbia River before reaching the Pacific Ocean. Although direct mortality at the dams has decreased in recent years (Muir et al. 2001), subsequent survival from out-migration until adult return is substantially lower for wild and hatchery Snake River spring

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Chinook salmon relative to some mainstem Columbia River populations (Berggren et al. 2008; Yakama Nation 2008). It has thus been hypothesized that delayed mortality may occur after passage out of the hydropower system (i.e., the impounded section of the rivers) because of the cumulative stress caused by dam passage (e.g., Budy et al. 2002; Schaller and Petrosky 2007). Therefore, to quantify delayed or indirect effects of dam passage, survival must be measured downstream of the final dam and preferably beyond fresh water.

The Pacific Ocean Shelf Tracking (POST) array (Welch et al. 2002) is a potentially continental-scale marine tracking array composed of individual acoustic receivers anchored to the ocean floor in a precise geometry. Each receiver records the date and time of uniquely coded acoustic tags passing nearby. In this study, cross-shelf ocean detection lines were located off southeast Alaska, British Columbia (around Vancouver Island), and Washington (40 km north of the Columbia River mouth), as well as in the Columbia and Snake rivers (Fig. 1). The potential tracking distance from the Snake River detection site to southeast Alaska was ~2300 km.

In 2006 we used the POST array to test the hypothesis that greater mortality is expressed in a population of hatchery-origin Snake River spring Chinook smolts after migrating through the impounded section of the river (eight dams) than that of a downstream population from the Yakima River (a tributary of the mid-Columbia River) that migrates through only four lower Columbia River dams. Smolt to adult return rates (SAR) of the Snake River population (Berggren et al. 2008) averaged only one-fifth that of the Yakima population (Yakama Nation 2008) for fish released between 1999 and 2005, and preliminary adult returns from the 2006 release year were consistent with prior years (SAR_{Yakima} = 2.7%; B. Bosch, Yakima–Klickitat Fisheries Project, 771 Pence Road, Yakima, WA 98908, USA, personal communication 2008; SAR_{Snake} = 0.38%, Berggren et al. 2008), providing a comparative test of whether this differential mortality develops shortly after they passed out of the hydrosystem. Our results provide the first estimates of survival and distribution in the ocean and substantially extend the period of life history during which is it possible to test whether delayed mortality occurs in juvenile salmon (Fig. 1).

Materials and methods

Populations used in the study

The Snake River population of spring Chinook used in this study was reared at the Dworshak National Fish Hatchery (NFH), on the Clearwater River (a tributary of the Snake River). Dworshak NFH spring Chinook from the Clearwater River are not a component of the Snake River evolutionary significant unit (ESU); however, their survival is routinely compared with other populations in the Snake River basin as well as with downstream Columbia River populations (see Berggren et al. 2008). Of the Snake River populations, Dworshak generally has the lowest SARs (Berggren et al. 2008), and therefore the difference in survival estimates should be particularly pronounced when compared with a population from outside of the Snake River basin. For logistical ease, Dworshak fish were transferred, tagged, and released at Kooskia NFH (60 km upstream of Dworshak NFH); distance to the Columbia River mouth is 870 km.

The Yakima population was reared at the Cle Elum Supplementation and Research Facility (CESRF) on the Yakima River. Migrating hatchery spring Chinook salmon released from CESRF acclimation sites were collected from the lower Yakima River at the Chandler Juvenile Monitoring Facility (CJMF; 194–249 km from the acclimation sites) and held for tagging. We collected fish at CJMF because mortality in the Yakima River has been as high as 80% in recent years (Yakama Nation 2008). Fish released from CJMF migrated 76 km down the Yakima River to enter the Columbia River just upstream from the Snake River – Columbia River confluence; distance to the Columbia River mouth is 615 km. Thus, both the Snake River and Yakima River populations shared a common migration path that passed through the four lower Columbia River dams before reaching the ocean listening line at Willapa Bay, a total distance of 910 and 655 km, respectively.

Tagging

We surgically implanted 794 smolts (two release groups of approximately 200 at each hatchery) with individually identifiable Vemco V9-6L acoustic transmitters (9 mm × 20 mm, 3.1 g in air, 2 g in water; Shad Bay, Nova Scotia, Canada). The tags were programmed to transmit a unique code at a mean interval of 60 s, and the rated battery life of the tag (4 months) was intended to exceed the likely migration time to the POST subarray in Alaska. A passive integrated transponder (PIT) tag was placed in the body cavity (through the incision) to ensure that tagged smolts were diverted back into the river at the dam bypass facilities. We set a minimum body size requirement of 140 mm fork length (FL), which meant that only the upper portion of the smolt size distribution could be tagged. The mean percent body weight of the transmitters was 8.3% (range = 2.6%–11.5%). The surgical procedure used to implant acoustic transmitters was approved by the Vancouver Island University Animal Care Committee.

Array location

Component subarrays were deployed within the Columbia River at McGowan's Channel at river kilometre (rkm) 224 (10 km below Bonneville Dam), in Lake Celilo at rkm 340 (7 km below John Day Dam), and in Lake Wallula at rkm 502 (21 km below the confluence of the upper Columbia and Snake rivers; Fig. 1). In the Snake River, receivers were installed in Lake Bryan at Snake River rkm 159 (14 km below Lower Granite Dam; total distance from the ocean was 681 km). At each of these locations, receivers were deployed as paired lines to assist in evaluating detection probability and to provide a survival measurement below three of the major hydroelectric dams.

Oceanic components of the POST array at Willapa Bay (southern Washington), Lippy Point (northwest Vancouver Island, British Columbia), and Graves Harbor (southeast Alaska) were deployed as single lines and extended from nearshore to the edge of the continental shelf (200 m depth), a distance of up to 30 km (Fig. 1).

Fig. 1. Panel (*a*) shows the 2006 Pacific Ocean Shelf Tracking (POST) acoustic array (black dots and thick black lines). The continental shelf (depths < 200 m) is shaded. Panel (*b*) shows the location of the release sites (CJMF, Chandler Juvenile Monitoring Facility; KNFH, Kooskia National Fish Hatchery), detection sites (McG, McGowan's Channel; LaC, Lake Celilo; LaW, Lake Wallula; LaB, Lake Bryan), and dams (BON, Bonneville; TDA, The Dalles; JDA, John Day; MCN, McNary; ICH, Ice Harbor; LMO, Lower Monumental; LGO, Little Goose; LGR, Lower Granite) within the Yakima, lower Snake, and Columbia rivers. Pie charts represent the proportion of Chinook salmon (*Oncorhynchus tshawytscha*) surviving to each detection site (Dworshak, grey; Yakima, black). Panels (*c*) and (*d*) show the cross-shelf distribution of Snake River and Yakima River smolts on the Willapa Bay line, respectively. Numbers above the bars represent the percentage of time the receiver was operational during the migration.

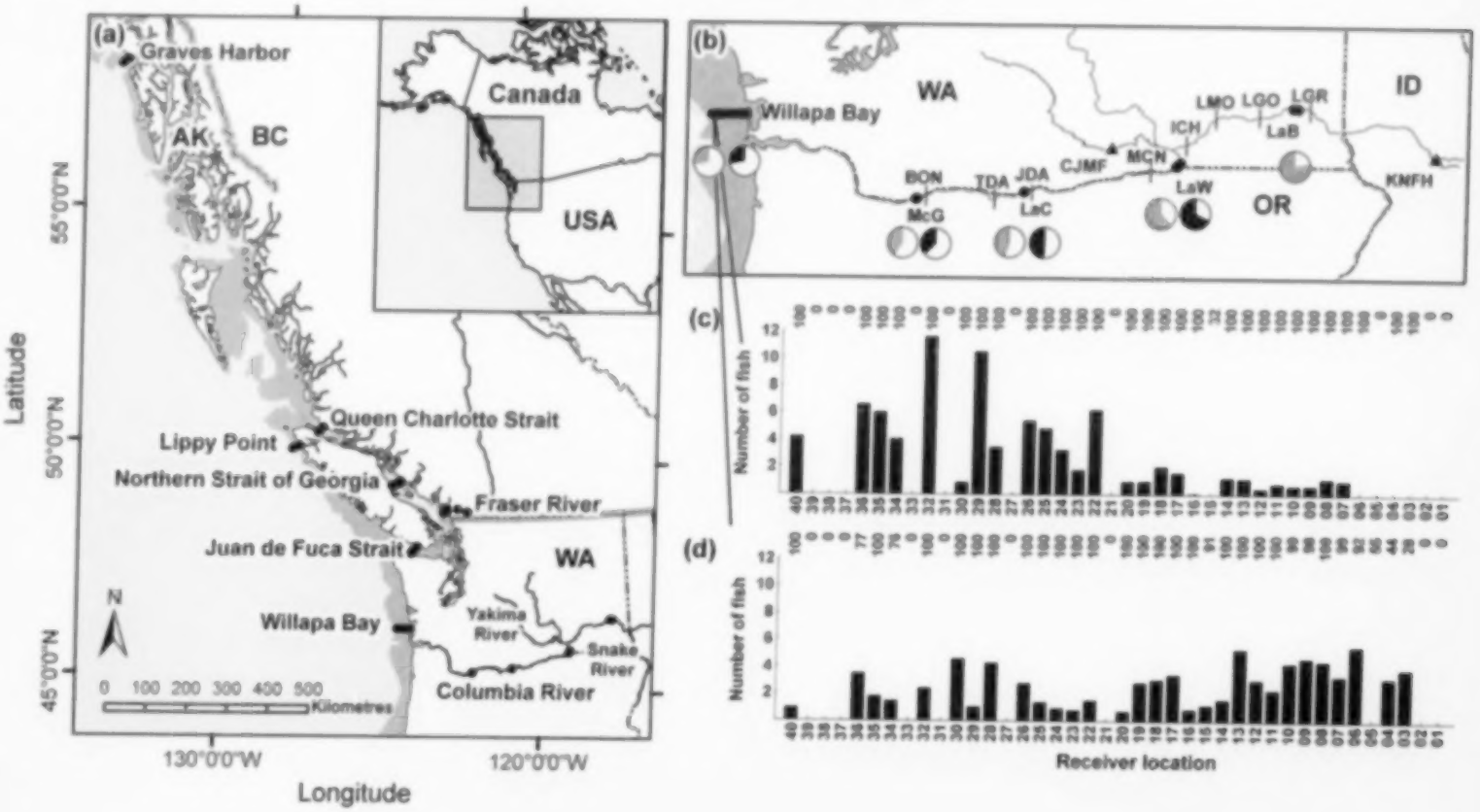

Table 1. Mean survival for Snake River (Dworshak) and Yakima River (Cle Elum Supplementation and Research Facility (CESRF)) spring Chinook salmon (*Oncorhynchus tshawytscha*) implanted with V9-6L acoustic transmitters and passive integrated transponder (PIT) tags in 2006.

Detection site	Segment distance (km)	Dams passed per segment	Est. *n*	Survival probability	Cumulative survival	Detection probability
Snake River						
LaB	189	1	308	0.78±0.02	0.78±0.02	0.97±0.02
LaW	179	3	245	0.78±0.02	0.61±0.03	0.92±0.03
LaC	162	2	176	0.79±0.02	0.48±0.04	0.56±0.06
McG	116	2	162	0.82±0.06	0.40±0.05	0.72±0.07
WiB	263	0	109	0.74±0.08	0.29±0.04	0.69±0.08
Yakima River						
LaB	NA	NA	NA	NA	NA	NA
LaW	113	0	258	0.70±0.03	0.70±0.03	1.00±0
LaC	162	2	186	0.72±0.03	0.51±0.03	0.77±0.05
McG	116	2	139	0.70±0.02	0.36±0.04	0.86±0.05
WiB	263	0	106	0.78±0.10	0.28±0.05	0.69±0.08

Note: Segment distance to the first detection site is measured from release location; all others are measured from previous detection site (LaB, Lake Bryan; LaW, Lake Wallula; LaC, Lake Celilo; McG, McGowan's Channel; WiB, Willapa Bay). Survival estimates are ±standard error (SE); NA, not applicable. Snake River smolts were released on 1 May (N = 198; mean fork length (FL) = 146.9 mm, range = 140–208 mm) and 8 May (N = 198; mean FL =145.6 mm, range = 140–192 mm); Yakima River smolts released on 30 May (N = 199; mean FL = 154.5 mm, range = 140–173 mm) and 6 June (N = 199; mean FL = 154.5 mm, range = 140–168 mm). The two release dates for Yakima River smolts were chosen in an attempt to have their arrival at Bonneville Dam coincide with the Snake River release groups. Yakima River smolts entered the Columbia River upstream of confluence with Snake River and therefore were not detected on the Lake Bryan line in the Snake River.

Survival analysis

Estimates of smolt survival (Φ) and detection probability (p) at each detection site were calculated using the Cormack–Jolly–Seber (CJS) model for live recaptured animals implemented in Program MARK (White and Burnham 1999). This model jointly estimates survival and detection within a likelihood framework. Because sample size beyond Willapa Bay was low, it was not possible to reliably estimate survival to British Columbia or Alaska. Therefore, we mainly focus on estimates of survival and detection to the ocean line at Willapa Bay.

For in-river lines, we recognized basic CJS model assumptions (equal survival probability, equal recapture probability, no tag loss, and instantaneous sampling); however, for oceanic lines that are not bounded on the offshore end (continental slope), we required two additional assumptions: (*i*) as fish migrate they cross over the acoustic detection lines that span the width of the continental shelf; and (*ii*) the majority of the fish departing the Columbia River swim north. These assumptions are supported by evidence from numerous ocean sampling programs (e.g., Fisher and Pearcy 1995; Brodeur et al. 2004; Bi et al. 2007).

To estimate overdispersion in the data, we used a median $\hat{c}$ procedure (White and Burnham 1999) to test the goodness of fit (GOF) of our global model ($\Phi_{\text{population} \cdot \text{line}} P_{\text{population} \cdot \text{line}}$) and then corrected for this overdispersion ($\hat{c}$ = 2.1) across all candidate models. We hypothesized that distance and number of dams within a migration segment (Table 1) may affect survival, so we modeled additive effects of each external covariate ($\Phi_{\text{population+distance}}$; $\Phi_{\text{population+dam number}}$), as well as the additive effects of both covariates ($\Phi_{\text{population+distance+dam number}}$), in addition to the fully varying CJS global model. Because of the low number of fish detected at Vancouver Island and Alaska, we included in our models two additional treatment groups (each N = 100) of spring Chinook smolts from Dworshak NFH, tagged with the same acoustic tag, that were released below Bonneville Dam during the spring out-migration to better quantify the detection probability of the Willapa Bay detection line. The survival estimate of this group was modeled in the same way across models ($\Phi_{\text{group} \cdot \text{line}}$) and did not influence survival estimates for run-of-river smolts. Recapture parameters were modeled to vary with population and line, except at Willapa Bay where all populations were pooled.

We used an information theoretic approach (i.e., Akaike's information criterion (AIC)) to select the most parsimonious model. In general, the model with the lowest AIC value (which accounts for the number of model parameters, n_{par}) has more support in the data, and if the ΔAIC of the other candidate models is greater than 2, then these models (i.e., hypotheses) have little or no support. We report survival estimates from the model that had the most support.

Cross-shelf distribution

For each population we used a χ^2 GOF test to determine if the cross-shelf ocean distribution adjacent to Willapa Bay was significantly different from the null hypothesis of a uniform distribution. To perform the tests, we counted the number of unique ID codes detected at each of the 40 receivers for each population. If a fish was detected at more than one receiver, a proportion was allocated to the receiver (e.g., if an ID code was detected on two receivers, each receiver was assigned a value of 0.5). If a receiver was displaced during the migration (e.g., by fishing activity), then that receiver was omitted from the data set (30 active receivers were used in the final analysis). To meet the requirements of the χ^2 test, we pooled data into 15 groups.

Results and discussion

Survival

The most parsimonious model based on an information theoretic approach was the model that constrained survival solely as a function of distance travelled ($\Phi_{population \cdot distance}$, AIC = 1677.0, n_{par} = 14). Although this model had considerably more support than the global model ($\Phi_{population \cdot line}$, AIC = 1687.7, ΔAIC = 10.7, n_{par} = 21), the added effect of distance on survival was negative for Dworshak smolts ($\Phi \cdot km^{-1}$ = 0.9993) as expected, but positive for the Yakima population ($\Phi \cdot km^{-1}$ = 1.0007). This was a surprising result for the Yakima population but was likely caused by low survival in the lower Yakima River (the shortest migration segment, which also lacked dams), combined with higher survival in the lower Columbia River and ocean (the longest segment). The model that constrained survival as a function of dam number also had support in the data ($\Phi_{population \cdot dam\ number}$, AIC = 1677.2, ΔAIC = 0.2, n_{par} = 14); however, the rate of survival per dam was slightly >1 for both populations, indicating that there was no negative survival effect due to the number of dams passed in a migration segment. The lack of effect of the number of dams passed on direct in-river survival may be a reflection of the improvements of fish passage in the hydrosystem (Muir et al. 2001), so that any remaining negative effect of the dams may be too small to be measurable once the effect of distance is accounted for. There was little support for a model that constrained survival as a function of number of dams within a migration segment and distance travelled ($\Phi_{population \cdot distance \cdot dam\ number}$, AIC = 1680.5, ΔAIC = 3.5, n_{par} = 16).

Survival of Snake River smolts ranged between 74% and 82% for individual river segments and between 70% and 78% for Yakima smolts (Table 1). Survivorship of Snake and Yakima smolts to McGowan's Channel, located just below Bonneville Dam (the final dam), was 40% and 36%, respectively. There was no detectable difference in survivorship from release to Willapa Bay between populations (S_{Snake} = 29%, S_{Yakima} = 28%; Table 1), and the mean time for smolts to reach this line after passing Bonneville Dam was ~5 days for both. Therefore, delayed mortality was not evident after several days in the coastal ocean at a point located 274 km beyond the hydrosystem. Although we tagged only larger individuals, and migration time was delayed by several weeks relative to untagged runs, these survivorship estimates were very similar to independent PIT tag estimates of survival of Dworshak (see Welch et al. 2008) and Yakima (D. Lind, Yakima–Klickitat Fisheries Project, 771 Pence Road, Yakima, WA 98908, USA, personal communication 2007) spring Chinook salmon in 2006.

Tagged Chinook salmon were also detected on acoustic lines on the west coast of Vancouver Island and Alaska. The large distance between ocean detection sites (~500 and 1000 km, respectively), the relatively low numbers detected on these lines, and the fact that the detected smolts arrived at both locations within 2 days of line deployment prevented us from reliably estimating survival to these locations. However, the detection of four Snake and two Yakima smolts on the Vancouver Island line, some 1500 km distant from the release site (30 days travel time from Willapa Bay for both populations), and the detection of two Snake River smolts on the Alaska line (and none from the Yakima population), some 2500 km from the release site, is inconsistent with the delayed mortality hypothesis.

Coastal ocean distribution

The cross-shelf distribution of the Snake River population was significantly different from the null hypothesis of a uniform distribution (χ^2 GOF test; $p < 0.01$), while the Yakima population was not ($p = 0.57$; Figs. 1*a*, 1*b*), with the Snake population more concentrated on the offshore portion of the line.

Study limitations

Several factors should be considered when interpreting our data. (*i*) We were limited to tagging smolts >140 mm FL, (*ii*) tagged fish were released 2–4 weeks after the main hatchery releases, (*iii*) tagged smolts were detected on the outermost receiver at Willapa Bay, suggesting that some smolts may migrate beyond the offshore extent of the subarray, and (*iv*) arrival to the first ocean line was not simultaneous for the two populations despite our attempts to do so. Future work should address these limitations by using smaller tags, extending the marine components of the array farther offshore (to depths ≥ 200 m), and by modifying release strategies to better synchronize release dates and arrival times in the ocean.

Conclusions

Lower river and early marine survival data for Columbia River basin Chinook salmon is essential for assessing the hypothesized delayed effects of dam passage on smolt survival. In this study, we found no evidence that delayed mortality was expressed by Snake River smolts (>140 mm FL) by the time they had reached the first ocean subarray, some 274 km from Bonneville dam, when compared with a size-matched group from the Yakima River population. This result is based on the assumption that smolts turn north after ocean entry and is supported by the high proportion of smolts detected on the Willapa Bay line subsequent to passing the detection line below Bonneville Dam. The mean time of arrival from below Bonneville Dam to Willapa Bay (5 days) puts an initial lower limit on the period when differential mortality may be expressed in these populations. Our results imply that if delayed mortality causes the disparity in adult return rates of hatchery-origin Snake River spring Chinook salmon, it is at a time and place more distant from the Columbia River; however, it is plausible that delayed mortality may operate on smaller fish (<140 mm FL) that were not tested here. Our result that the number of dams passed within a migration segment did not affect survival also supports the findings that direct in-river survival of salmon smolts has increased in recent decades (Muir et al. 2001), possibly to the level of an undammed river (Welch et al. 2008). We note that our study was not designed to measure very small effects on mortality, as it was focused on making the first large-scale measurements of migratory survival for salmon originating from the Columbia River. It is not correct to conclude that the dams currently play no role in affecting survival, simply that any contributory effect appears to be small.

The differing marine distributions of the two populations at Willapa Bay provides a potential mechanism in support of the alternative hypothesis that populations from the same river basin can have different responses to ocean conditions (e.g., Levin 2003). It remains unclear whether the entire size range of these two populations, as well as wild smolts, have similar survival and behavior as the smolts reported here. The technical development of the POST array allowed us to make a direct scientific test of a key hypothesis concerning delayed mortality of a population of Snake River spring Chinook salmon in the ocean. As such, it marks an important scientific milestone. Advances in the array design, however, will allow us to study marine survival over the complete size range of naturally occurring spring Chinook smolts, as well as other endangered populations of salmon originating from the Columbia River basin.

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Reduced swimming performance and increased growth in domesticated rainbow trout, *Oncorhynchus mykiss*

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Abstract: Domesticated populations of rainbow trout (*Oncorhynchus mykiss*) probably have been selected for high growth rates and large body size, which may have resulted in reduced sprint swimming performance. We hypothesized that more domesticated populations of rainbow trout would have higher growth rates and larger body size, but slower swim speed, relative to semiwild populations. We tested this hypothesis by measuring body mass, body length, and sprint swim speed multiple times over 92 days in progeny from crosses between males from three clonal lines and an outbred female. We found significantly higher body masses and significantly slower swim speeds in the highly domesticated Arlee and Hot Creek progeny groups compared with the semiwild Swanson hybrid progeny group, supporting our hypothesis. Growth rates also differed significantly among groups, but at the ages measured, the Swanson hybrid progeny had an intermediate growth rate. However, given the differences in body mass, either growth rates were higher in the more domesticated progeny groups at young unmeasured ages and (or) they hatched at a significantly larger body mass than the semiwild Swanson hybrid.

Résumé : Les populations domestiquées de truites arc-en-ciel (*Oncorhynchus mykiss*) ont probablement été sélectionnées pour leur fort taux de croissance et leur importante taille corporelle, ce qui peut avoir conduit à une performance réduite de la nage de pointe. Nous posons l'hypothèse selon laquelle les populations plus domestiquées de truites arc-en-ciel devraient avoir un taux de croissance plus élevé et une taille corporelle plus forte, mais une vitesse de nage plus lente, par comparaison aux populations semi-sauvages. Nous avons testé cette hypothèse en mesurant la masse corporelle, la longueur du corps et la vitesse de nage de pointe à de nombreuses reprises sur une période de 92 jours chez des rejetons de croisements entre des mâles de trois lignées clonales et une femelle exogame. La masse corporelle est significativement plus forte et la vitesse de nage significativement plus lente chez les groupes de rejetons des lignées fortement domestiquées Arlee et Hot Creek, par rapport au groupe de rejetons hybrides de la lignée semi-sauvage Swanson, ce qui confirme notre hypothèse. Les taux de croissance diffèrent aussi parmi ces groupes, mais aux âges où nous avons fait les mesures, les rejetons hybrides Swanson ont un taux de croissance intermédiaire. Cependant, compte tenu des différences de masse corporelle, il se peut que les taux de croissance aient été plus élevés dans les groupes de rejetons plus domestiqués aux âges antérieurs à nos mesures ou alors (et) que ces rejetons aient éclos avec une masse corporelle significativement plus grande que les hybrides semi-sauvages Swanson.

[Traduit par la Rédaction]

Introduction

In natural populations of animals, strong correlations between large body size and increased evolutionary fitness are frequently measured; large body size is related to increased fecundity, reduced predation, and, in general, an increased ability to survive in a given environment (Hone and Benton 2005). Such relationships between fitness and body size lead to the simple prediction that body size should be under strong directional selection, which in turn should lead to an erosion of the additive genetic variance for body size as alleles for large body size become fixed (Falconer and Mackay 1996). However, this prediction is not supported by many studies that have demonstrated high levels of additive genetic variance for body size in a variety of animals (review in Mangel and Stamps 2001).

The fact that so much genetic variance exists for a trait that should be under strong directional selection suggests the possibility of evolutionary trade-offs between body size or growth rate and other components of Darwinian fitness (Mangel and Stamps 2001). This idea is supported by a variety of evidence. For example, a number of studies have found that within species, including Atlantic salmon (*Salmo salar*) (McDonald et al. 1998), rainbow trout (*Oncorhynchus mykiss*) (Gregory and Wood 1998), Atlantic silverside (*Menidia menidia*) (Billerbeck et al. 2001), largemouth bass

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(*Micropterus salmoides*) (Kolok 1992), anurans (Arendt 2003), and salamanders (Fitzpatrick et al. 2003), faster-growing animals exhibit reduced locomotor performance compared with slower-growing animals. Similarly, there is evidence that aggression, foraging, reproduction, and antipredator behaviors are inversely related to growth rate when comparing farmed fish with their wild counterparts (Huntingford 2004).

In fish hatcheries, both inadvertent and purposeful selection have taken place for high growth rates (Kincaid 1994; Petersson and Jarvi 2000; Fleming et al. 2002), which has led to increased body size and growth rate in many hatchery populations (Fleming and Einum 1997; Molony et al. 2004; Tymchuk and Devlin 2005). However, fast-growing domesticated fish may suffer increased mortality under natural conditions because of increased foraging exposure to predators and diminished antipredator behavior (Tymchuk et al. 2007). An underlying performance component of predator avoidance is sprint swimming speed (Walker et al. 2005). Because sprint swim speed is likely to be crucial to the survival of hatchery rainbow trout released into the wild, we wanted to test whether domestication results in slower-swimming trout. We hypothesized that domesticated rainbow trout would exhibit a larger body size, but reduced sprint swimming performance, relative to wild rainbow trout. We tested this hypothesis utilizing clonal rainbow trout from three different source stocks. Use of clonal lines has the advantage of limiting genetic variance within groups and maximizing it among groups but comes with the cost of utilizing only a small portion of the available genetic variation from within each source stock; regardless, use of this system of clonal trout has been fruitful in identifying genetic components of phenotypic variation (e.g., Robison et al. 1999; Lucas et al. 2004; Nichols et al. 2004). The Hot Creek (California) and Arlee (Montana) clonal lines were derived from stocks that have been under domestication for over 100 years and are considered to be fully domesticated, whereas the Swanson (Alaska) clonal line has only been in domestication for two to three generations and is considered to be semiwild (Young et al. 1996; Robison et al. 1999). Thus, we hypothesized that progeny from the domesticated Hot Creek and Arlee lines would show increased body size but decreased sprint swim performance relative to progeny from the Swanson line.

Materials and methods

Study organisms

Clonal rainbow trout (*O. mykiss*) were used as the male parents in this study. Clonal rainbow trout lines are homozygous, genetically uniform, and propagated by androgenesis (induced all-paternal inheritance) or gynogenesis (induced all-maternal inheritance) (reviewed in Young et al. 1996). The female parent in this study was a single outbred, domesticated female from Troutlodge Hatchery, Soap Lake, Washington. Twenty-one outbred male (all male due to doubled haploid YY parent) juvenile rainbow trout were randomly selected from each of the crosses between the outbred female and the three clonal trout lines (Hot Creek, Arlee, and Swanson hybrid) for a total of 63 trout. Thus in producing the outbred male progeny, the sperm from one clonal doubled haploid male was used from each line and the egg source was the same outbred female for all individuals. Each rainbow trout group was fertilized the same day, and all progeny were placed in progeny group tanks at swim up on the same day in the same approximate densities (~50 per tank). At 90 days after ponding (i.e., after the initiation of feeding), all individuals from all progeny groups were randomly placed in individual tanks (230 mm × 110 mm × 115 mm) in a 10 °C environmental chamber on a 14 h light : 10 h dark cycle in Eastlick Hall at Washington State University. Individual tanks were cleaned and water was exchanged with dechlorinated water three times a week. Standard trout meal was provided once a day.

Because of an error in strain identification, the Swanson line utilized by this study was in fact a clonal hybrid cross of the Oregon State University (OSU) female and Swanson male lines and so is referred to as the "Swanson hybrid" line herein. The OSU is another highly domesticated clonal line maintained at Washington State University (WSU) derived from the Shasta type rainbow trout lines. While still a clonal line, this means the Swanson hybrid line was 50% Swanson (semiwild) and 50% OSU (domesticated) in genetic origin. Therefore, our experimental test fish in the Swanson progeny group were 25% semiwild and 75% domesticated in origin, while the Hot Creek and Arlee groups were 100% domesticated in origin.

Sprint swimming tunnel

We utilized a phototransistor sprint swimming tunnel based on a design by Nelson et al. (2002); measuring burst or sprint swimming speed using a still-water tunnel and either phototransistors or stop-action cameras is now routine (e.g., Billerbeck et al. 2001; Nelson et al. 2002; Arendt 2003). The swimming tunnel was 100 cm long × 10 cm wide × 13 cm high and made of clear Plexiglas. We used laser pointers powered by a constant current as a light source for creating a plane of light the animal had to pass through in the sprint swimming tunnel. To spread the laser light, the laser beam was passed through a 0.6 cm diameter glass rod. On the opposite side of the swimming tunnel from each light source, a vertical bank of 12 phototransistors were set up in series in line with the spread laser beam. A 9 V battery was used to supply a constant voltage across each bank of phototransistors. A detectable change in voltage across the phototransistors is caused by a change in resistance when a transistor is interrupted as an animal swims by and blocks the light source. A total of three banks of phototransistors were set 10.2 cm apart along the length of the swim tunnel. Voltage was sampled by a Sable data acquisition system (200 samples·s^{-1}), saving all files as time-indexed ASCII files, which were then processed using a custom SPSS program that calculated speed (cm·s^{-1}) for each animal involved (SPSS Inc., Chicago, Illinois).

Trait measurements

Measurements of body mass and total body length were taken at 90 days after ponding and every day on which a sprint swimming speed measurement was taken. Sprint swimming speed was measured four times, once every 21 to 28 days over a 92-day period. For each day of measurements, individuals were randomized with regard to order of

measure and time of day; time of day was recorded for use as a statistical covariate. Prior to each measurement, all individuals were fasted for 24 h. Fish tanks were not marked with the group identity of the fish, and the numbering scheme to identify individuals was randomized so that group identity could not be inferred from individual identification number; hence, the experimenter was blind with regard to the group identity of each individual. On each measurement day, each individual was tested five times consecutively, with the fastest speed between any two points in the tunnel chosen as the maximum sprint swimming speed. To motivate maximum sprint swimming speed, individuals were lightly tapped in the caudal area with a glass rod for the duration of the trial (Nelson et al. 2002); if the investigator was unsuccessful doing this for a given trial, the data from that trial was discarded. The same investigator swam all fish for all trials throughout the experiment.

Statistical analysis

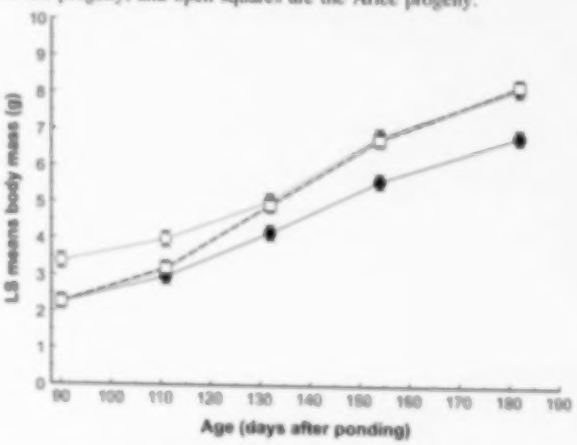
Relative growth rate (RGR) was calculated as $[\ln(\text{mass}_2 - \text{mass}_1)]/(\text{day}_2 - \text{day}_1)$ (Kingsolver et al. 2001), and the condition factor (K) was calculated as (mass × 100 000)/(length^3). Sprint swim speed, body mass, body length, RGR, and K factor were subjected to repeated measures analysis of variance (ANOVA) using PROC MIXED in SAS (SAS Institute Inc., Cary, North Carolina). In all analyses, individual was the repeated variable and was modeled as a random effect, the Satterweith option was used to estimate the correct degrees of freedom, and the covariance structure was modeled using the spatial power law to account for higher covariances among measures closer together in time (Littell et al. 1996). In any analysis with a significant ($p < 0.05$) progeny group by day interaction, contrast statements were used to test for differences in the linear, quadratic, and cubic components of the progeny group by day interaction curves to identify specific differences in curves. Contrast coefficients were estimated using PROC IML in SAS.

Sprint swim speed was initially modeled using progeny group, day, progeny group by day interaction, body mass, body mass by progeny group interaction, tank location, and time of day of measure. (Body mass was used as the size covariate instead of length because body mass is more physiologically relevant than length in studies of locomotion (reviewed in Willmer et al. 2005) and because the model using body mass fit the data better than the model with length using Akaike's information criterion; in addition, an unpresented analysis using body length as the size covariate gave similar results.) Because the body mass by progeny group interaction was not significant ($p < 0.05$), it was removed from the model and sprint swim speed reanalyzed using the remaining variables. Body mass, RGR, body length and condition factor were modeled using progeny group, day, progeny group by day, and tank location. Dependent variables were transformed as needed to meet the assumptions of normality required for the mixed model analysis.

Results

Body mass by age after ponding for each progeny group is illustrated (Fig. 1). Differences among progeny groups in body mass were statistically significant in the repeated measures analysis (Table 1; $p = 0.0002$). The Swanson hybrid progeny had the lowest average body mass across all ages and was significantly different from the Hot Creek (Table 1; $p = 0.0001$) and the Arlee (Table 1; $p = 0.0124$) progeny using Tukey pairwise comparison tests. The Hot Creek and Arlee progeny did not differ in body mass (Table 1; $p = 0.2308$). The day by progeny group interaction was also significant (Table 1; $p < 0.001$), indicating differences among progeny groups in the shapes of the body mass curves. The Swanson hybrid and Hot Creek progeny groups have similarly shaped body mass curves compared with the Arlee progeny, which had a more rapid increase in mass (Fig. 1). Contrast statements testing for differences in linear, quadratic, and cubic components of the curves revealed significant differences between the Arlee progeny group and the Swanson and Hot Creek progeny groups in the linear component of the curves (Table 1; $p < 0.0001$ in both comparisons) and between the Arlee and Hot Creek curves in the quadratic component (Table 1; $p = 0.0445$). Day was also statistically significant (Table 1; $p < 0.001$), demonstrating the expected result that all fish increased body mass with increasing age. Finally, tank location was a significant covariate (Table 1; $p = 0.0144$), indicating that tank location on the rearing racks had a significant effect on body mass. Because tank location was randomized among progeny groups and was used as a statistical covariate, this did not affect the overall conclusions of the experiment.

Fig. 1. Body mass least squares (LS) means and standard errors (from the repeated measures analysis) for all three progeny groups of rainbow trout (*Oncorhynchus mykiss*) by day of measure. Solid circles are the Swanson hybrid progeny, open circles are the Hot Creek progeny, and open squares are the Arlee progeny.



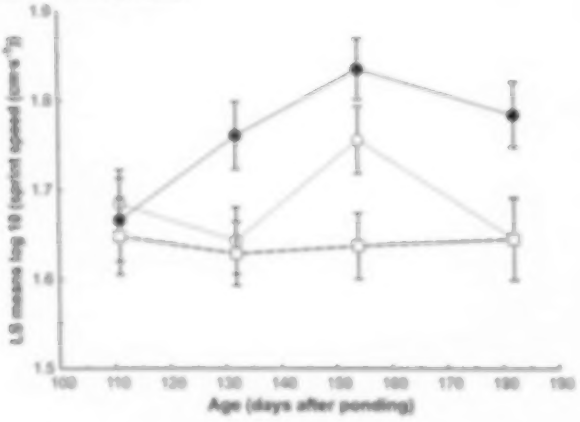
Differences in sprint swim speed were tested using a repeated measures analysis that included body mass and time of day of measure as covariates. Initial models also included the body mass by progeny group interaction term to test for differences in the body mass – sprint swim speed slopes among progeny groups; this term was not statistically significant and so was dropped from the final analysis. The final analysis revealed significant differences in sprint swimming speed among progeny groups (Table 1; $p = 0.0005$; Fig. 2); Tukey pairwise comparisons identified differences between

Table 1. Significance (p) values from mixed model repeated measures analysis for each trait.

Main effects	Body mass	Sprint speed	RGR	Length	K factor
ProgenyGroup	**0.0002**	**0.0005**	**<0.0001**	**<0.0001**	**<0.0001**
Day	**<0.0001**	0.0932	**<0.0001**	**<0.0001**	**<0.0001**
ProgenyGroup × Day	**<0.0001**	0.0623	**<0.0001**	**<0.0001**	**<0.0001**
Location	**0.0144**	0.3511	**0.0366**	0.0772	0.1681
Mass	—	**0.0038**	—	—	—
Time	—	**0.0225**	—	—	—
Tukey					
HC vs. SW	**0.0001**	**0.0432**	**0.0079**	**0.0184**	**<0.0001**
HC vs. AR	0.2308	0.3131	**<0.0001**	**0.0009**	**<0.0001**
SW vs. AR	**0.0124**	**0.0003**	**0.0276**	0.5880	**<0.0001**
Shape contrasts					
Linear					
HC vs. SW	0.1875	—	**0.0001**	**0.0290**	**0.0066**
HC vs. AR	**<0.0001**	—	**<0.0001**	**<0.0001**	**<0.0001**
SW vs. AR	**<0.0001**	—	**0.0543**	**0.0013**	0.0559
Quadratic					
HC vs. SW	0.1911	—	0.0737	0.3683	**<0.0001**
HC vs. AR	**0.0445**	—	0.1167	0.0513	**<0.0001**
SW vs. AR	0.4774	—	0.8184	0.2957	**0.0010**
Cubic					
HC vs. SW	0.4371	—	**0.0241**	0.8178	0.2789
HC vs. AR	0.7804	—	**0.0003**	0.6847	0.3574
SW vs. AR	0.2936	—	0.1539	0.5238	0.8815

Note: RGR, relative growth rate; HC, Hot Creek; AR, Arlee; SW, Swanson. A dash indicates exclusion of an effect or a test from a model. Values less than 0.05 are in bold.

Fig. 2. Sprint swim speed least squares (LS) means and standard errors (from the repeated measures analysis) for all three progeny groups by day of measure. Solid circles are the Swanson hybrid progeny, open circles are the Hot Creek progeny, and open squares are the Arlee progeny.



the Swanson hybrid progeny group and both the Hot Creek (Table 1; $p = 0.0432$) and the Arlee (Table 1; $p = 0.0003$) progeny groups, with no differences between Arlee and Hot Creek (Table 1; $p = 0.3131$). The interaction term between progeny group and day was not significant (Table 1; $p = 0.0623$); hence, no significant differences in curve shape among the three progeny groups were identified. Body mass was a significant covariate in this analysis (Table 1; $p = 0.0038$). The relationship between body mass and swimming speed for each day for each progeny group is shown (Fig. 3). Within each group, sprint swim speed tended to increase with increasing body mass; however, the position of the Swanson regression curve relative to those of the other two progeny groups is higher for 3 of the 4 days. Finally, time of day of measure was a significant covariate for sprint swim speed (Table 1; $p = 0.0225$).

Repeated measures analysis of RGR showed significant differences among all three pairs of progeny groups (Table 1; main effect $p < 0.0001$; Hot Creek vs. Swanson $p = 0.0079$; Hot Creek vs. Arlee $p < 0.0001$; Swanson vs. Arlee $p = 0.0276$), with the Swanson hybrid progeny being intermediate between the Hot Creek and Arlee progeny at younger ages but with all three progeny groups converging at older ages (Fig. 4). A significant progeny group by day interaction was also identified (Table 1; $p < 0.0001$), with all the Hot Creek progeny differing from the other two progeny groups in both the linear (Table 1; Hot Creek vs. Swanson $p = 0.0001$, Hot Creek vs. Arlee $p < 0.0001$) and cubic components of the curves (Table 1; Hot Creek vs. Swanson $p = 0.0241$, Hot Creek vs. Arlee $p = 0.0003$). These results indicate differences among progeny groups in both the position and shapes of the growth curves such that the progeny groups significantly differed in their growth rates and also in how growth rate changed with age. The covariate tank position was also significant (Table 1; $p = 0.0366$).

Body length showed a significant effect of progeny group using repeated measures analysis (Table 1; $p < 0.0001$), which is being driven by significant differences between the Hot Creek progeny and the Swanson (Table 1; $p = 0.0184$)

Fig. 3. Raw sprint speed by body mass for all three progeny groups presented separately for each of the four measurement days. Panel *a* is 111 days after ponding, panel *b* is 132 days after ponding, panel *c* is 154 days after ponding, and panel *d* is 182 days after ponding.

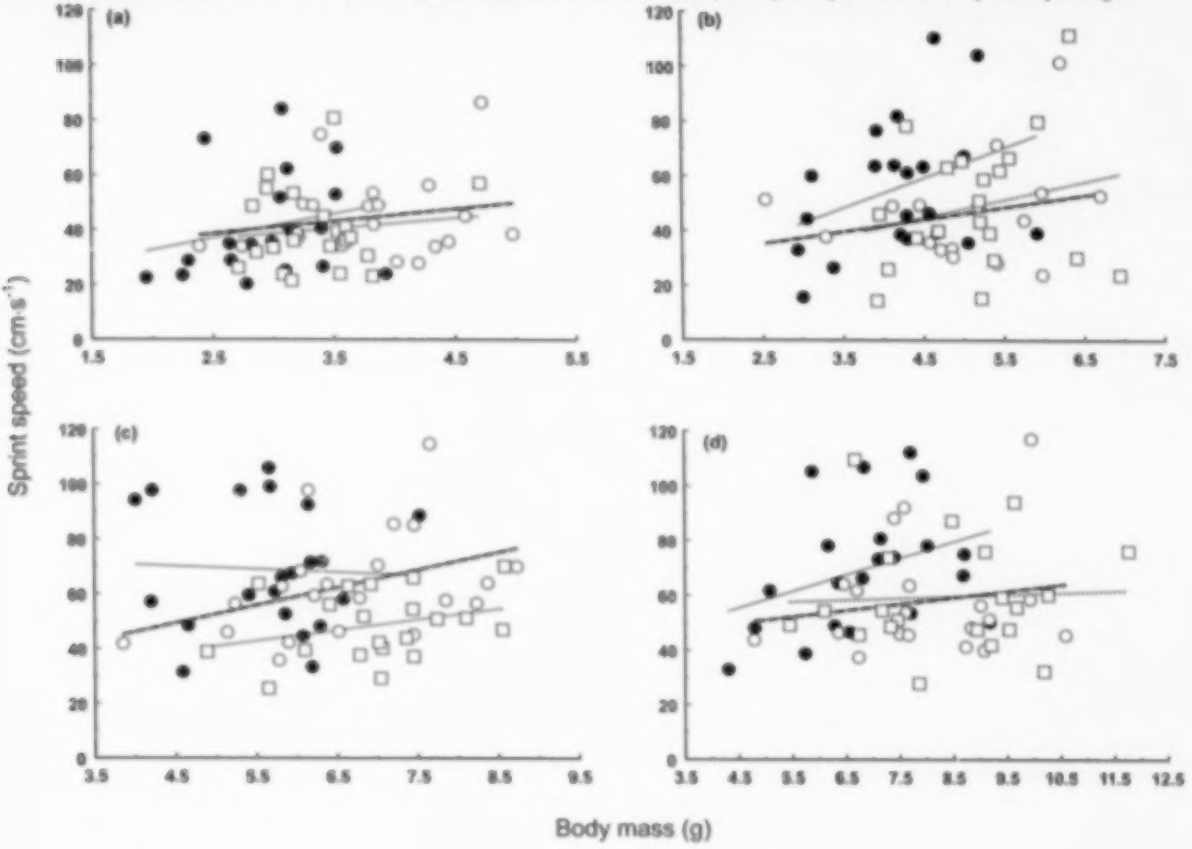
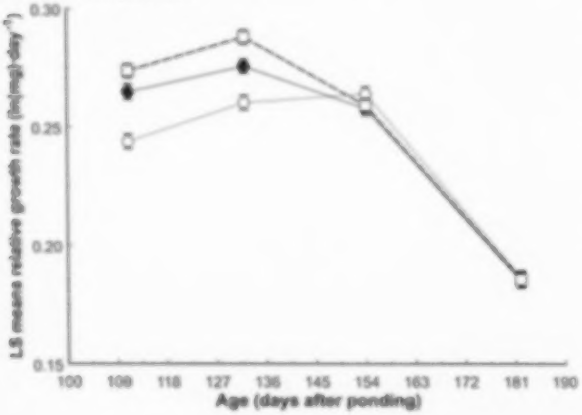


Fig. 4. Relative growth rate least squares (LS) means and standard errors (from the repeated measures analysis) for all three progeny groups by day of measure. Solid circles are the Swanson hybrid progeny, open circles are the Hot Creek progeny, and open squares are the Arlee progeny.



and Arlee (Table 1; $p = 0.0009$) progeny groups. A significant progeny group by day interaction was also found, with all three pairs of progeny groups differing in the linear component of the curve (Table 1; Hot Creek vs. Swanson $p = 0.0078$, Hot Creek vs. Arlee $p < 0.0001$, Swanson vs. Arlee $p = 0.0006$) and the Hot Creek and Arlee differing in the quadratic component ($p = 0.0477$). Thus, body length curves differ in both position and shape, with Hot Creek having the longest length at all ages and Arlee having the shortest length at younger ages but increasing in length more rapidly at older ages than the Swanson hybrid progeny (Fig. 5).

The condition factor, K, was also significantly different among progeny groups (Table 1, $p < 0.0001$), with all three pairs of progeny groups differing from each other at the 0.0001 level (Table 1). A significant progeny group by day interaction was identified (Table 1, $p < 0.0001$), with Hot Creek differing from Swanson (Table 1; $p = 0.0066$) and Arlee (Table 1; $p < 0.0001$) in the linear component and all three pairs of progeny groups differing in the quadratic component (Hot Creek vs. Swanson $p < 0.0001$, Hot Creek vs. Arlee $p < 0.0001$, Swanson vs. Arlee $p = 0.0010$). Swanson had the lowest K value at all ages, and Arlee had the highest K value at all ages (Fig. 6).

Fig. 5. Body length least squares (LS) means and standard errors (from the repeated measures analysis) for all three progeny groups by day of measure. Solid circles are the Swanson hybrid progeny, open circles are the Hot Creek progeny, and open squares are the Arlee progeny.

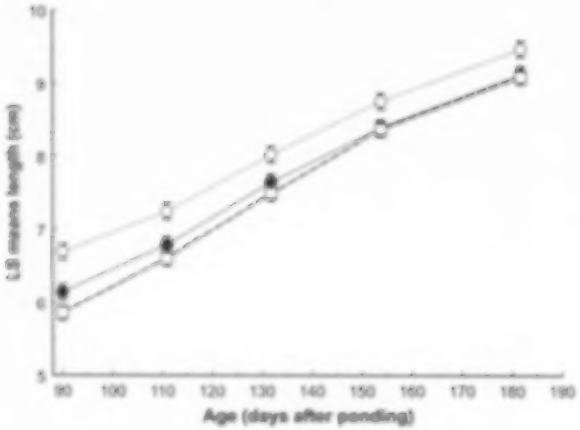
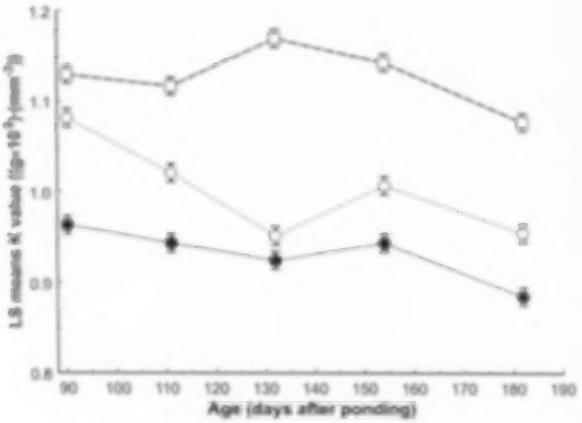


Fig. 6. *K* value least squares (LS) means and standard errors (from the repeated measures analysis) for all three progeny groups by day of measure. Solid circles are the Swanson hybrid progeny, open circles are the Hot Creek progeny, and open squares are the Arlee progeny.



Discussion

Our results support the hypothesis that more domesticated lines of rainbow trout are poor swimmers relative to wilder strains. The semiwild Swanson hybrid progeny had significantly higher sprint swim speeds across all ages compared with the fully domesticated Arlee and Hot Creek progeny groups. This result is particularly notable considering that the Swanson hybrid progeny had only 25% semiwild ancestry. Body mass also significantly differed as predicted among the progeny groups, with the semiwild Swanson hybrid progeny having a lower body mass at all ages than the more domesticated Arlee and Hot Creek progeny groups. Therefore, our results support the hypothesis of trade-offs between body size and swim performance in domesticated rainbow trout. Interestingly, within a given group, swim speed increased significantly with increasing body mass. This suggests significant genetic differences among groups but continuing genetic variation for size and speed within groups. Such a result argues strongly that quantitative trait locus (QTL) studies to identify chromosomal regions that influence both sprint swimming and body mass in rainbow trout would be fruitful. QTLs for early-age body mass have already been identified (Drew et al. 2007).

The results with regard to growth rate appear to be somewhat more complicated. At the ages measured, the semiwild Swanson hybrid had an intermediate growth rate at younger ages, with differences in growth rate among the groups dissipating as the fish got older. However, given the differences measured in body mass, either growth rates were higher in the more domesticated progeny groups at very young (and unmeasured) ages, or they hatched at a significantly larger body mass than the semiwild Swanson hybrid, or both. Hence, given the differences in body mass among groups, it is highly likely that growth rates differed in the same direction as body mass at younger, unmeasured ages.

The semiwild Swanson progeny group had the lowest condition factor and had the highest sprint swim speeds. This suggests that although high condition factor may be a good economic trait in aquaculture, it is not necessarily a good indicator of physical or evolutionary fitness for fish in natural environments.

The differences in body mass, swimming performance, and condition factor among progeny groups measured herein must have a genetic basis. Fish from all groups were raised in a "common garden" both before and during the measurement period; therefore, no consistent environmental effects can be causing the measured among-group differences (Falconer and Mackay 1996). In addition, genetic variance was high among progeny groups and low within progeny groups because each progeny group was produced by mating a homozygous (doubled haploid) clonal male to the same outbred female. On average, then, individuals within progeny groups shared 75% of the same alleles, whereas no restriction was imposed on among-progeny group genetic variation. Such carefully controlled environmental and genetic conditions strongly suggest that the trade-off between growth rate and sprint swimming ability has a genetic basis; however, it is important to keep in mind that only a limited amount of the available genetic variation within a source stock is contained within a clone. A number of other traits, such as development rate (Robison et al. 2001), immune response (Zimmerman et al. 2004), disease resistance (Nichols et al. 2003), and stress response (Drew et al. 2007) have already been shown to have a genetic basis in clonal line crosses.

Relationships between growth rate and sprint swimming ability similar to those described herein have been reported in other aquatic species. Arendt (2003) found a trade-off between rapid growth rates and sprint swimming speed in two species of anuran tadpoles, American toad tadpoles (*Bufo americanus*) and western spadefoot tadpoles (*Spea hammondii*). Billerbeck et al. (2001) and Lankford et al. (2001) found a negative correlation between growth rate and sprint swimming ability between the northern (increased growth,

decreased sprint swimming ability) and southern (decreased growth, increased swimming ability) populations of the Atlantic silverside (*Menidia menidia*). These studies also used a common-garden experimental design, indicating a likely genetic basis for the differences in growth rate and swimming ability. Finally, Gregory and Wood (1998) found a significant negative correlation between specific growth rate and relative U_{crit} values (a measure of maximal sustained swimming speed, not sprint speed) in food-restricted rainbow trout, supporting the idea that fast-growing fish exhibit reduced swimming ability. That study, however, did not establish a genetic basis to the relationships between swimming performance and growth rate.

High growth rate is a commercially valuable trait because less money and time will be spent to produce the same amount of biomass from faster-growing individuals relative to slower-growing individuals. The same arguments can be used to justify rapid growth in hatcheries intended to enhance fisheries. Growth rate and large body size is easily selected under domestication in fish hatcheries (Thorpe 1991; Jonsson et al. 1996; Quinn et al. 2004). However, the results presented herein demonstrate a crucial trade-off, which appears to be genetically based, between size and swimming performance that could potentially lead to the erosion of evolutionary fitness of hatchery populations. This may be especially critical for hatchery populations that are released into the wild to fend for themselves; hence, breeding programs that de-emphasize selection on size, or that allow for the regular introgression of "wild" genes, might be crucial for the long-term success of hatchery populations. The relationship that we describe may account for some of the erosion in survival of anadromous salmonids that has been reported with progressive generations of culture in hatcheries (Araki et al. 2008).

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Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA

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Abstract: The ratio of RNA to DNA (RNA:DNA) in white muscle tissue of juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) was used as a proxy for recent growth rate in an estuarine nursery. Variability in RNA:DNA was examined relative to temporal changes in temperature and dissolved oxygen (DO). Initial laboratory experiments indicated (*i*) a strong positive relationship between RNA:DNA and growth rate, (*ii*) a rapid response of RNA:DNA to changes in feeding, and (*iii*) no effect of hypoxia on the relationship between RNA:DNA and growth rate (tested in weakfish only). Diel cycling DO occurred in the nursery throughout the summers of 2002 and 2003. Canonical correlation analysis of field data indicated a strong positive relationship between RNA:DNA and mean DO conditions prior to capture in both species. Correlations were weak or insignificant between stomach content mass (an index of feeding) and mean DO and between RNA:DNA and stomach content mass and DO variability. These results suggest a strong functional relationship between DO concentration and the growth rate of juvenile fishes in an estuarine nursery. Furthermore, growth rates of wild-caught fishes (estimated from RNA:DNA) appear to be more negatively impacted by diel cycling hypoxia than would be expected from published laboratory data.

Résumé : Le rapport ARN sur ADN (ARN:ADN) dans le tissu du muscle blanc du cardeau d'été (*Paralichthys dentatus*) et de l'acoupa royal (*Cynoscion regalis*) a servi de mesure de remplacement du taux de croissance récent dans une nourricerie estuarienne. Nous avons examiné la variabilité d'ARN:ADN en fonction des changements temporels de température et d'oxygène dissous (DO). Des expériences initiales en laboratoire indiquent (*i*) une forte relation positive entre ARN:ADN et le taux de croissance, (*ii*) une réaction rapide de ARN:ADN aux changements d'alimentation et (*iii*) une absence d'effets de l'hypoxie sur la relation entre ARN:ADN et le taux de croissance (testée seulement chez l'acoupa royal). Il y a eu un cycle journalier de DO dans la nourricerie tout au cours des étés 2002 et 2003. Une analyse des corrélations canoniques des données de terrain montre une forte relation positive entre ARN:ADN et les conditions moyennes de DO avant la capture chez les deux espèces. Les corrélations sont faibles ou insignifiantes entre la masse du contenu stomacal (un indice alimentaire) et la valeur moyenne de DO et entre ARN:ADN et la masse du contenu stomacal et la variabilité de DO. Ces résultats indiquent une forte relation fonctionnelle entre les concentrations de DO et le taux de croissance des jeunes poissons dans une nourricerie estuarienne. De plus, les taux de croissance des poissons capturés en nature (estimés d'après ARN:ADN) semblent être affectés plus négativement par l'hypoxie cyclique journalière que ne le laissent prévoir les données de laboratoire de la littérature.

[Traduit par la Rédaction]

Introduction

Growth rate during the early life stages of marine and freshwater fishes is thought to influence survival and recruitment. In most cases, faster growth is believed to benefit survival of larval and juvenile fishes (but see Billerbeck et al. 2001 and Lankford et al. 2001). Subtle decreases in growth rate prolong stage duration (Houde 1987) and increase the susceptibility to size-selective predation and overwinter mortality (see review by Sogard 1997). Hence, habitats that promote rapid growth and increased survival of juvenile fishes, often termed nurseries, are considered to be critical to the success of coastal fisheries (Beck et al. 2001). In the US, such habitats have been deemed essential fish habitat, which is broadly defined by the Magnuson–Stevens Act of 1996 as those waters and substrata necessary to fish for spawning, breeding, feeding, or growth to maturity.

Estuaries, coastal bays, and their tributaries constitute important nursery habitats during the larval and juvenile stages of many estuary-dependent fishes (Weinstein 1979; Hoss and Thayer 1993; Minello et al. 2003). The functional value of these habitats as nurseries is attributed primarily to physicochemical regimes (e.g., temperature and salinity) that are physiologically suitable or optimal, abundant prey resources,

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and low predation risk: conditions that promote rapid growth and enhance survival of young fishes (Joseph 1973; Miller et al. 1985; Gibson 1994). The anthropogenic deterioration of water quality, however, seriously threatens the nursery value of these habitats. More specifically, coastal eutrophication and associated hypoxia is of growing concern because of potentially negative impacts on fishes and their habitats (Diaz 2001; Kennish 2002; Diaz and Rosenberg 2008).

The incidence and severity of hypoxic events in coastal waters has risen worldwide during the last century (see reviews by Diaz and Rosenberg 1995; Diaz 2001; Diaz and Rosenberg 2008). By definition, hypoxia is any dissolved oxygen (DO; mg $O_2 \cdot L^{-1}$) concentration less than saturation (~7 mg $O_2 \cdot L^{-1}$). DO concentrations in shallow estuarine and marine systems are generally between air saturation and 2 mg $O_2 \cdot L^{-1}$ (Diaz and Rosenberg 1995). Currently, the US Environmental Protection Agency (USEPA 2000) maintains a 4.8 mg $O_2 \cdot L^{-1}$ growth protective DO criteria and a 2.3 mg $O_2 \cdot L^{-1}$ survival protective DO criteria for the most sensitive species in the saltwater faunal community (fishes and crustaceans) in the Middle Atlantic Bight (US east coast).

Whereas chronic hypoxia and anoxia are common in the deep waters of seasonally stratified systems such as the Chesapeake Bay (Officer et al. 1984; Breitburg 1990), shallower habitats routinely undergo severe diel (over 24 h) fluctuations in DO (from 0% to 300% saturation over 24 h) in response to photosynthesis and respiration cycles of phytoplankton and macroalgae (D'Avanzo and Kremer 1994; Tyler et al. 2009). In these environments, hypoxia is predominantly a warm-water phenomenon, occurring during spring and summer when stratification is greatest (Welsh and Eller 1991). The timing of these hypoxic conditions corresponds to the period of high juvenile abundance in the estuarine nurseries of the Middle Atlantic Bight (Weinstein 1979; Able and Fahay 1998), and consequently, estuary-dependent fish communities are likely to be adversely affected by hypoxia.

To date, the identification and description of functional relationships between environmental conditions and variability in the recruitment of marine organisms has been somewhat limited (Anderson 1988; Rose 2000; Menge et al. 2002). Given the deterioration of DO conditions in coastal habitats, it is increasingly important to understand the potential consequences for growth and survival of estuary-dependent fishes. It is generally accepted that severe hypoxia results in mortality. Surprisingly, however, little evidence exists for the sublethal impacts of hypoxia on growth of fishes in the wild.

Previous laboratory studies indicate that sublethal levels of chronic and diel cycling hypoxia can limit growth rates (e.g., Chabot and Dutil 1999; Pichavant et al. 2001; Stierhoff et al. 2006) and thus potentially decrease the value of hypoxic waters as nurseries. Although laboratory studies provide valuable information on the relative sensitivity of various species and functional groups to hypoxia, constraints imposed on behavior and other experimental artifacts in such studies can limit extrapolation of results to fish responses in natural systems. For instance, estuarine fishes are capable of detecting and avoiding hypoxic conditions (Wannamaker and Rice 2000; Eby and Crowder 2002; Bell and Eggleston 2005), which could mitigate the effects of low DO on growth in the wild. In such cases, intermittent moderate hypoxia may in fact augment fish growth by enhancing foraging success on stunned or moribund prey upon their return following improved DO conditions (Pihl et al. 1992; Breitburg 2002). Conversely, avoidance of hypoxic water may crowd fishes into more oxygenated areas, thereby increasing the likelihood of density-dependent growth limitation (Pihl et al. 1991; Eby and Crowder 2002). The effects of hypoxia on growth are further complicated by varying behavioral strategies of coping with hypoxia in fishes with different life history strategies (e.g., sedentary, demersal vs. active, pelagic fishes) (Schurmann and Steffensen 1994; Dalla Via et al. 1998; Wannamaker and Rice 2000).

Our rather limited understanding of the effects of hypoxia on growth of wild fishes in estuarine systems is likely due to the temporally and spatially dynamic nature of DO and the difficulty of measuring growth on physiologically and ecologically relevant time scales. Caging has been used to compare somatic growth of fishes between different habitats (Sogard 1992; Phelan et al. 2000; Meng et al. 2004) and physicochemical regimes (Meng et al. 2000, 2001; Eby et al. 2005). However, caging studies are labor intensive and suffer to an unknown degree from artifacts including handling stress, accumulation or exclusion of prey items, and (or) the restriction of natural movement (Able 1999; Meng et al. 2000), all of which could confound the response of growth rates to the environmental parameter(s) of interest. Microstructural analysis of hard parts (e.g., otoliths) as a proxy for short-term growth rates may also be useful (see Rakocinski et al. 2006), but may also lack the temporal resolution over the time scales of interest in hypoxia studies.

Alternatively, nucleic acid-based indices, including RNA concentration ([RNA]), [RNA]:protein, [RNA]:tissue mass, and RNA:DNA have been used extensively as a proxy for physiological condition (feeding and growth rate) of larval (e.g., Buckley 1980, 1984; Caldarone et al. 2003) and juvenile fishes (e.g., Malloy and Targett 1994; Rooker and Holt 1996; Peck et al. 2003). Of these, RNA:DNA is most commonly used. DNA content of fish tissues remains relatively constant and provides an index of cell number, while RNA content changes in response to transcription-dependent protein synthesis, which is directly correlated with ribosomal activity and thus growth rate (Bulow 1970). The efficacy of RNA:DNA lies in its ability to estimate short-term growth rates (i.e., is sensitive to changes in growth in as little as 24 h in summer flounder, *Paralichthys dentatus*; Malloy and Targett 1994) of free-ranging individuals while avoiding many of the problems associated with the caging approach. Coupled with frequent fish sampling and continuous water quality monitoring, RNA:DNA-based growth rate estimates could greatly improve our ability to resolve relationships between recent growth and environmental factors, such as DO, that vary over small spatial and temporal scales.

In this study, we measured RNA:DNA as a proxy for growth rate to examine spatial and temporal variation in physiological condition of free-ranging juvenile weakfish (*Cynoscion regalis*) and summer flounder (*P. dentatus*) in response to changes in physicochemical conditions in Pepper Creek. These estuary-dependent species represent two distinct life histories: weakfish, an actively swimming benthopelagic fish, and summer flounder, a demersal flatfish. Pepper Creek is a shallow, mesohaline tidal creek tributary

to Indian River Bay, Delaware Coastal Bays (USA). Pepper Creek exhibits major temporal (both diel and seasonal) and spatial variability in DO, temperature, and salinity (Tyler et al. 2009). Furthermore, newly settled juvenile summer flounder and weakfish are abundant in Pepper Creek between April and September when physicochemical conditions show the greatest variability. Therefore, a model system exists to examine growth responses under changing environmental conditions.

Preliminary laboratory experiments were necessary to validate the utility of RNA:DNA as an accurate and temporally sensitive proxy of growth rate for these fishes under such conditions. These experiments described (*i*) the relationship between RNA:DNA and growth rate over a range of temperatures for each species, (*ii*) the temporal response of RNA:DNA to diet-induced changes in growth rate for each species, and (*iii*) the potentially confounding effect of hypoxia on the linear relationship between RNA:DNA and growth rate. Next, fish sampling and DO monitoring were conducted at three locations in Pepper Creek, between May and September in 2002 and 2003. Growth rates of juvenile summer flounder and weakfish from field collections were measured using RNA:DNA, and changes in growth were compared with changes in environmental conditions.

Materials and methods

Laboratory growth experiments

Juvenile (young of the year) summer flounder and weakfish were collected from Pepper Creek between April and August 2002 (Fig. 1). In both years, summer flounder recruited earlier and were most abundant between May and July, whereas weakfish recruited somewhat later and were most abundant between July and August. Prior to acclimation, fish were maintained at a 14 h light : 10 h dark photoperiod (light period 0700–2100 in all experiments) and fed frozen mysid shrimp, *Mysis relicta* (average ± standard error (SE) mysid mass and length were 0.032 ± 0.020 g and 16.03 ± 3.34 mm, respectively; $n = 20$), ad libitum twice daily. All fish were acclimated to experimental temperatures (see below) and salinity (25‰ for summer flounder and 20‰ for weakfish) for ≥10 days.

Relationship between RNA:DNA and growth rate

Since temperature has been shown to influence the relationship between RNA:DNA and growth rate (Buckley et al. 1999), summer flounder (62–95 mm standard length (SL); 3.5–14 g) and weakfish (56–81 mm SL; 3–10 g) were grown at four temperatures. Temperature treatments for summer flounder (18, 22, 26, and 30 °C) and weakfish (20, 23, 27, and 30 °C) were chosen to encompass the potential range of temperatures experienced by these two species in Pepper Creek during the field portion of the study. Somatic growth rate (in mass and length) was measured in individual fish after 7 days and regressed on the RNA:DNA measured in the white muscle tissue of each fish at the end of the experiment (see below).

To achieve a range of growth rates at each temperature, ration was manipulated in three food treatments: ad libitum feeding, restricted ration, and starved. Fish were held individually in 8 L containers in filtered, recirculating water. Fish were fed twice daily in ad libitum treatments and once daily in restricted ration treatments (summer flounder received 8 mysids; weakfish received 10 mysids in the restricted ration treatment). Temperature and ration treatments followed a fully crossed 4 × 3 temperature–ration ($n = 5$ per treatment; $n = 60$ total per experiment) experimental design. Food was withheld for 18 h prior to initial mass and length measurements to minimize the effect of stomach contents on growth rate estimates, but not before final weighing to be certain to preserve any differences in RNA:DNA between feeding treatments. Immediately after final mass and length measurements, each fish was killed by a sharp blow to the head, rapidly frozen on dry ice, and stored at –80 °C for subsequent RNA:DNA analysis. Stomachs were removed from the thawed fish, and the mass of stomach contents in fed treatments were subtracted from final mass of the fish prior to calculating growth rate. Instantaneous growth rate (G, day^{-1}) of each fish was calculated as $G = (\ln M_f - \ln M_i) \cdot d^{-1}$; where M_f is final wet mass, M_i is initial wet mass, and d is the number of days. Specific growth rate (SGR, % body mass·day^{-1}) was calculated as $\mathrm{SGR} = (e^G - 1) \times 100$ (Ricker 1975).

Temporal response of RNA:DNA to changes in feeding and SGR

For each species, 85–90 fish (66–118 mm SL for summer flounder; 52–89 mm SL for weakfish) were held in a ~220 L tank (0.6 m width × 2.5 m length × 0.15 m diameter) at 25 °C and fed frozen *M. relicta*, ad libitum twice daily (0800 and 1500) for ≥10 days, after which five fish were randomly sampled from the population at 1500 on each of three consecutive days (see Fig. 2). At 1500 on day 3, food was removed and all fish were held without food for the next 5 days. Beginning at 1500 h on day 3, five fish were sampled randomly at 6 h intervals (2100, 0300, 0900, and 1500) for the next 24 h, then at 1500 every 24 h each day through to day 8. At 1500 on day 8, food was re-introduced and again, five fish were sampled randomly at 6 h intervals for the next 24 h and at 1500 daily thereafter until no fish remained (through to day 12). Fish from each species were serially sampled from a population held in a single tank (all individuals experiencing the same environmental conditions) to avoid environmental differences associated with holding fish in multiple tanks. Each fish thus provided an independent estimate (replicate) at specific time points to assess the population's temporal response (in RNA:DNA) to changes in feeding. Immediately after sampling, each fish was killed by a sharp blow to the head, frozen rapidly on dry ice, and stored at –80 °C for subsequent quantification of nucleic acids.

Effect of DO on the relationship between RNA:DNA and growth rate

To examine the potential effect of hypoxia on the RNA:DNA–growth rate relationship, RNA:DNA and SGR were measured in juvenile weakfish (51–75 mm SL; $n = 10$ per treatment) held individually for 7 days at 25 °C under ad libitum feeding conditions and exposed to 7 mg $O_2 \cdot L^{-1}$ (i.e., normoxia) or one of three constant hypoxia treatments (2.0, 3.5, 5.0 mg $O_2 \cdot L^{-1}$). Since growth rates of weakfish are generally unaffected above 5 mg $O_2 \cdot L^{-1}$ (Stierhoff et al. 2009),

Fig. 1. Location of sampling sites in upper (1), middle (2), and lower (3) Pepper Creek, Indian River Bay, Delaware Coastal Bays (location in eastern USA shown in inset).

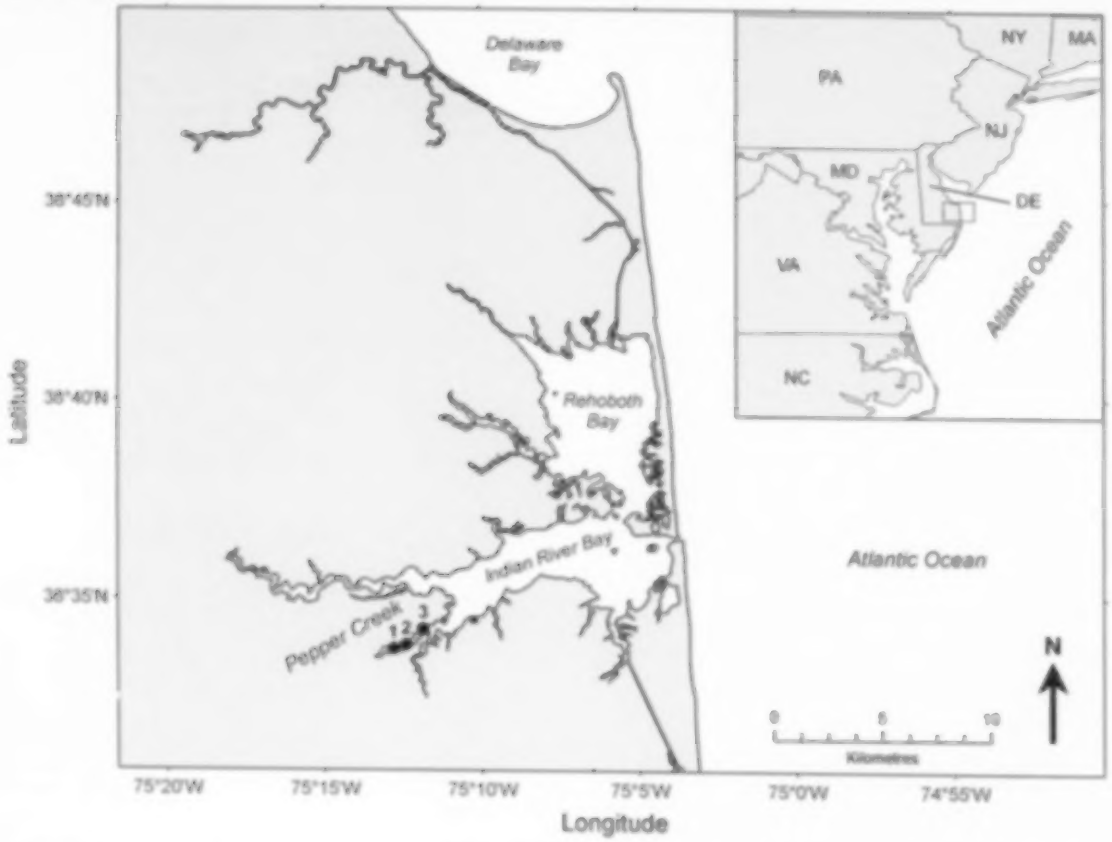
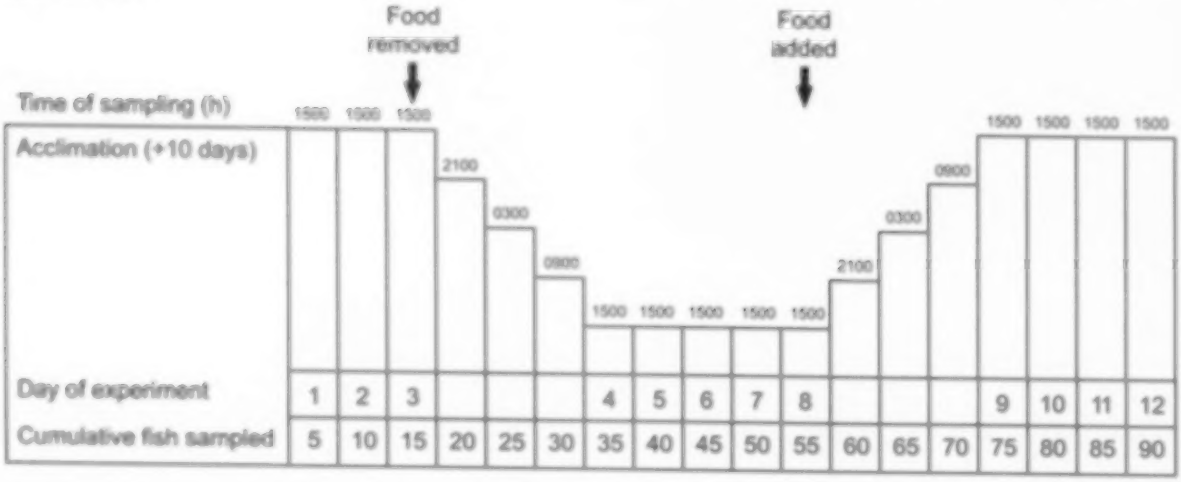


Fig. 2. Feeding and sampling protocol used to determine the temporal dynamics of RNA:DNA in response to changes in feeding. Fish were acclimated for ≥10 days prior to beginning sampling on day 1. Feeding ceased on day 3 and resumed on day 8. Time of sampling is indicated above each bar.



fish were grouped into high (5 and 7 mg $O_2 \cdot L^{-1}$) and low (2 and 3.5 mg $O_2 \cdot L^{-1}$) DO treatments. SGR was regressed on RNA:DNA and DO to examine the relationship between SGR and RNA:DNA, as well as any potential effect that different DO treatments had on this relationship. DO was regulated using the computer-controlled device described by Grecay and Stierhoff (2002). Fish were fed frozen *M. relicta* ad libitum twice daily. Immediately after final weighing and measuring, each fish was killed by a sharp blow to the head, frozen rapidly on dry ice, and stored at –80 °C for subsequent quantification of nucleic acids.

Quantification of nucleic acids

A subsample of white muscle tissue was dissected from the epaxial musculature above the lateral line of each frozen fish. Frozen, but not freeze-dried, muscle samples were sonicated and digested in 2% *N*-lauroylsarcosine (sarcosil) to dissociate nucleoproteins. Aliquots of these preparations were diluted with Tris–EDTA buffer to a final concentration of 0.1% sarcosil before quantifying nucleic acids.

Nucleic acid content of these preparations was quantified using the one dye – two enzyme (ethidium bromide (EB) plus RNase and DNase) microplate fluorometric assay developed for larval fish and described by Caldarone et al. (2001). Fluorescence was read at excitation and emission wavelengths of 520 and 612 nm, respectively. Some modifications to the assay were made for use with white muscle tissue of juvenile fishes (E. Caldarone, National Marine Fisheries Service – NOAA, 28 Tarzwell Drive, Narragansett, RI 02882, USA, unpublished protocol). In particular, black 96-well Costar nonbinding surface plates (NBS, Costar 3650) were used to minimize fluorescence quenching (decrease in fluorescence of DNA standards following the addition of RNase) observed in preliminary assays.

Total fluorescence of standards and samples were measured at 30 °C after addition of EB using a BMG Labtechnologies POLARstar OPTIMA fluorometer. Subsequently, RNA and DNA fluorescence was measured as the decrease in fluorescence following sequential digestion with RNase and DNase, respectively. Residual fluorescence (~30%; attributed to factors other than RNA or DNA) remained after digestion with both enzymes. Standard calibration curves were constructed using serial dilutions of 18s- and 28s-rRNA (Sigma R-0889) and calf thymus DNA (Sigma D-4764) standards. Concentrations of rRNA standard curves ranged from 3.30 to 11.22 $\mu g \cdot mL^{-1}$, and DNA standard curves ranged from 0.48 to 2.35 $\mu g \cdot mL^{-1}$. Recovery rates of known mixtures of RNA and DNA standards were 92% and 103%, respectively. The slope ratio of DNA and RNA standard curves (mDNA/mRNA) was 2.645 ± 0.121 (n = 20 sample runs).

Statistical analyses

Least squares multiple linear regression analysis was used to describe the relationship among SGR and RNA:DNA, temperature, and body mass of juvenile summer flounder and weakfish. Least squares linear regression analysis was also used to determine the rate of change in RNA:DNA following food removal and refeeding. The time at which RNA:DNA differed significantly from fully fed and starved values was determined using analysis of variance (ANOVA) and Tukey's multiple comparison test comparing RNA:DNA across time intervals. Data used in both the regression and ANOVA analyses were normally distributed (determined by Kolmogorov–Smirnov test using the Lilliefors option), and variances were homoscedastic. Finally, least squares multiple linear regression analysis was used to describe the relationship between SGR and RNA:DNA of juvenile weakfish across DO treatments. All statistical analyses were conducted using SPSS v. 11.5 (SPSS Inc., Chicago, Illinois; α = 0.05 for all analyses).

In situ growth study

Study site

Pepper Creek is a shallow (~0.5–3 m), tidal, mesohaline (15‰–20‰) tributary to Indian River Bay, Delaware Inland Bays (Fig. 1), that experiences spatially (tens of metres to kilometres) and temporally dynamic DO (over the diel cycle and seasonally), driven primarily by photosynthesis, temperature, and insolation (Tyler et al. 2009). Pepper Creek also supports high abundances of juvenile summer flounder and weakfish (Tyler 2004). Three sites were sampled along the axis of Pepper Creek from May to September in 2002 and 2003 (Fig. 1). The spatial extent of the sampling design was necessarily small (e.g., the distance between Sites 1 and 3 was ~1.65 km) to facilitate the frequent temporal sampling of fishes and maintenance of field equipment throughout each sampling season.

Physicochemical monitoring

Near-bottom (~15 cm above the sediment surface) temperature, salinity, and DO were measured at each site every 15 min for the duration of the study using Yellow Springs Instruments multiparameter sondes (models 600XLM and 6280). Sonde probes were serviced approximately weekly to prevent fouling of the DO sensor membrane and to maintain integrity of the environmental data. Because of the limited availability of sondes, physicochemical monitoring and fish sampling at Site 2 was not initiated until 2 July 2002.

In situ condition estimates

Juvenile summer flounder and weakfish were sampled from all three sites (except as noted above) every 3–4 days between May and September of 2002 and 2003, using a 3.5 m otter trawl (38 mm stretch mesh net, 25 mm stretch mesh cod end, 6 mm square mesh cod end liner, with ~5 m of chain attached to the foot rope). At least five individuals of each species were selected at random from the catch, killed immediately by a sharp blow to the head, frozen rapidly on dry ice, and stored in the laboratory at –80 °C for subsequent quantification of nucleic acids. In the laboratory, stomach contents were removed and weighed (g, wet weight) to provide an index of feeding.

Statistical analyses

The relationship between hypoxia and physiological condition of summer flounder and weakfish in Pepper Creek was examined in two ways. First, analyses of the correlations among RNA:DNA, stomach contents, and the DO conditions prior to fish capture were done using canonical correlation analysis. The mass of the fish's stomach contents was included in the analysis in an effort to discriminate whether oxygen conditions affected RNA content directly (i.e., through increased metabolic costs) or indirectly by affecting the amount of prey consumed by the fish. The ob-

Table 1. Multiple linear regression coefficients describing the relationship among specific growth rate (SGR, % body mass·day^{-1}), RNA:DNA (a_1), temperature (°C, a_2), and body mass (g, a_3) of juvenile summer flounder (*Paralichthys dentatus*, 62–95 mm standard length (SL); 3.5–14 g) and weakfish (*Cynoscion regalis*, 56–81 mm SL; 3–10 g) in the laboratory.

	Coefficient (±SE)					
	a_1	a_2	a_3	C	F ratio	Adjusted R^2
Summer flounder	0.733 (0.14)	0.240 (0.08)	–0.011 (0.11)	–11.784 (3.04)	11.225*	0.354
Weakfish	0.885 (0.11)	0.342 (0.06)	0.371 (0.11)	–17.597 (1.97)	36.381*	0.703

Note: SGR = $a_1 + a_2 + a_3 + C$, where C is a constant. Both models are significant ($p < 0.01$). All parameters included in the model were also significant ($p < 0.01$), except for body mass (a_3) in the case of summer flounder ($p = 0.92$). SE, standard error.

served mass of the fish's stomach contents at the time of capture scaled allometrically with fish mass. Consequently, to use an index of stomach content mass in the correlation analysis, the fish's stomach content mass and total body mass were logarithmically transformed, and then a linear regression line was fit to this data to express predicted (log) stomach content mass as a function of (log) fish mass for each species. The deviations (residuals) of the individual fish from this predicted line were then used as one of the variables as a way of controlling for the effect of fish size in the later analyses.

The sondes provided measurements, at 15 min intervals, of DO, salinity, and temperature. The mean DO (DO_{mean}), DO coefficient of variation (DO_{CV}), mean salinity, and mean temperature were calculated for a series of successively longer time spans preceding the collection of fish at that site (i.e., DO_{mean} for 1 h prior to collection, then the same statistics were calculated using data for the inclusive 2 h prior to fish collection, and so on) up to a time span of 48 h prior to fish collection. DO_{CV} values were examined to accommodate the possibility that fish RNA:DNA or stomach content mass were associated with the short-term variability in DO values.

It is possible that the four variates of interest (RNA:DNA, stomach content mass residual, DO_{mean} over a time span, and DO_{CV} over a time span) would also vary with respect to ambient temperature and salinity, and this would confound the canonical correlation analysis by affecting the rates of RNA synthesis or feeding. To control for salinity and temperature, multiple linear regressions were calculated, with one of the above four variates as the response variable and the mean temperature and salinity over the same time span as the explanatory variable. The residuals of the four variables from the values predicted by temperature and salinity were calculated and used in the canonical correlation analyses. Because of this, the correlations reported here would more properly be termed partial correlations, controlling for the effect of salinity and temperature.

Canonical correlation analysis examines two sets of variables for the correlations between them, and it can be considered that one set of variables are response variables, while the other set are explanatory variables. In this case, the set of response variables was the RNA:DNA values for each fish and the residual value of the log of stomach content mass. These two variables were collectively considered to represent fish condition at the time of collection. Canonical correlation analysis calculates a canonical variable as a composite of the observed variables and maximizes the correlation of this response canonical variable with a counterpart canonical variable calculated from the set of explanatory variables. In the analyses done here, only one explanatory variable was used: either DO_{mean} over a specified time period or DO_{CV} over a time period. In summary, 96 canonical correlation analyses were done for each species: 48 relating RNA content and stomach content mass to DO_{mean} values for the time spans of 1–48 h prior to fish capture and 48 analyses relating the same response variates to DO_{CV} over identical time spans. No statistical inferences are made regarding the correlation coefficients; rather they are viewed only as indicators of the association between fish condition measures and oxygen values.

The second way we examined the relationship between hypoxia and physiological condition involved using in situ SGR of weakfish and summer flounder from Pepper Creek calculated from RNA:DNA, temperature, and body mass using the multiple regression equations in Table 1. Then, predicted SGR of both species were calculated from DO and temperature measurements experienced prior to collection (see below). In situ SGR was plotted against predicted SGR to examine the observed responses of these fishes to hypoxia in the wild versus responses in laboratory experiments.

Growth rates of juvenile summer flounder and weakfish, calculated from prior laboratory experiments (Stierhoff et al. 2006, 2009), were used to model growth rates across a range of temperature and DO conditions. Predicted growth rates (SGR_{pred}) were then calculated for fishes collected from Pepper Creek using the growth equations in Table 2 and compared with back-calculated SGR estimates of the same individuals based on RNA:DNA. In these equations, T is the average temperature (°C), and DO is the average DO concentration (mg O_2·L^{-1}) measured every 15 min during the 24 h period prior to fish collection. Since body mass significantly affected the relationship between RNA:DNA and SGR for weakfish, these analyses were restricted to fishes within the size range used to develop the relationship between RNA:DNA and growth rate of each species in the laboratory portion of this study. Therefore, some larger summer flounder and smaller, newly settled weakfish were excluded from analyses in both years.

Results

Laboratory growth experiments

Relationship between RNA:DNA and growth rate

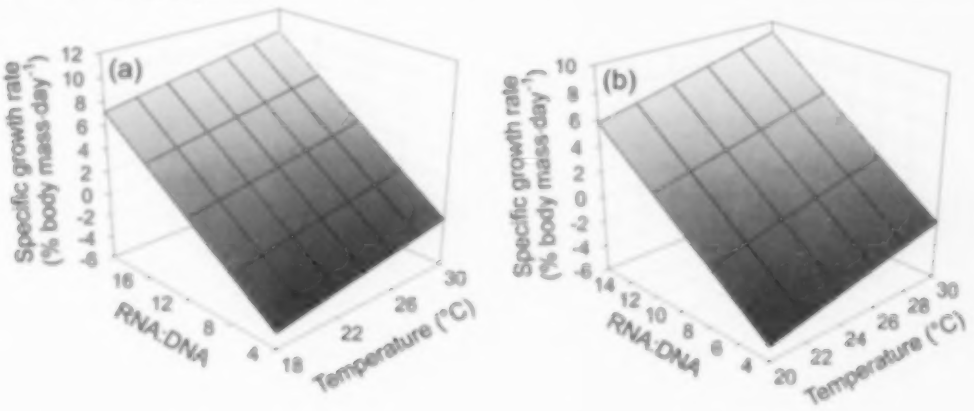
Mean SGR ranged from –3.54% to 7.37% body mass·day^{-1} for summer flounder and –2.90% to 8.46% body mass·day^{-1} for weakfish across all temperature and ration treatments. No mortality occurred in any treatments for either species.

Table 2. Regression coefficients used to calculate predicted specific growth rates (SGR_{pred}, % body mass·day^{-1}) of juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) in Pepper Creek from temperature (T, °C) and dissolved oxygen (DO, mg O_2·L^{-1}) measured prior to collection.

		Coefficient (±SE)					
	n	a_1	a_2	a_3	a_4	C	Adjusted R^2
Summer flounder	119	2.749 (0.461)	−0.056 (0.009)	2.315 (0.355)	−0.180 (0.039)	−35.081 (5.690)	0.697
Weakfish	119	−2.069 (0.936)	0.046 (0.019)	1.300 (0.721)	−0.130 (0.078)	23.471 (11.547)	0.160

Note: $SGR_{pred} = a_1 + a_2 + a_3 + a_4 + C$, where $a_1 = T$, $a_2 = T^2$, $a_3 = DO$, $a_4 = DO^2$, and C is a constant. Both models are highly significant ($p < 0.01$). SE, standard error.

Fig. 3. Relationship between RNA:DNA and specific growth rate of juvenile (*a*) summer flounder (*Paralichthys dentatus*) and (*b*) weakfish (*Cynoscion regalis*) at different temperatures.



There was a significant positive relationship between RNA:DNA and SGR of summer flounder (multiple regression analysis; $n = 57$, adjusted $R^2 = 0.354$, $p < 0.01$) and weakfish (multiple regression analysis; $n = 58$, $R^2 = 0.703$, $p < 0.01$) (Table 1, Fig. 3). Temperature had a significant effect on the relationship between RNA:DNA and growth rate of both species (Table 1). As temperature increased, SGR of both species was higher at a given RNA:DNA (Fig. 3). Body mass had a significant effect on the relationship between RNA:DNA and SGR in weakfish, but not in summer flounder (Table 1). There was no indication of multicollinearity (i.e., significant correlation of independent variables) in either analysis.

Temporal response of RNA:DNA to changes in feeding and SGR

RNA:DNA of both species exhibited a rapid (nearly instantaneous), linear response to changes in feeding status (Fig. 4). Following food removal, RNA:DNA of summer flounder decreased –0.63·day^{-1} and was significantly lower than fully fed levels within 48 h of the removal of food (ANOVA, $p < 0.05$) (Fig. 4*a*). Upon refeeding, RNA:DNA of summer flounder increased 0.91·day^{-1} and was significantly higher than the starved values within 48 h (ANOVA, $p < 0.05$). RNA:DNA of weakfish decreased faster than summer flounder (–1.94·day^{-1}) and was significantly lower than fully fed levels within 18 h of food removal (ANOVA, $p < 0.05$) (Fig. 4*b*). Upon refeeding, RNA:DNA of weakfish increased 1.19·day^{-1} and was significantly higher than starved values within 48 h (ANOVA, $p < 0.05$).

Effect of DO on the relationship between RNA:DNA and growth rate

Even under ad libitum feeding conditions, a wide range of weakfish growth rates were observed in each of the four DO treatments (Fig. 5). The relationship among SGR, RNA:DNA, and DO in this experiment is described by the following equation: SGR = 0.94(RNA:DNA) – 0.06(DO) – 5.16 (multiple linear regression; $n = 36$, adjusted $R^2 = 0.80$, $p < 0.01$). There was a significant positive relationship between SGR and RNA:DNA ($p < 0.01$), but no significant relationship between SGR and DO (high vs. low DO) ($p = 0.53$), indicating that the relationship between RNA:DNA and SGR was independent of DO concentration.

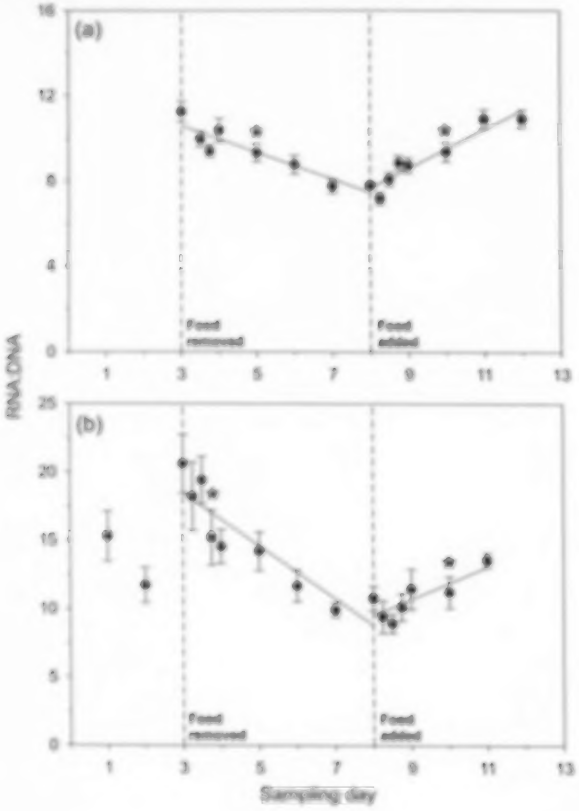
In situ growth study

Physicochemical conditions in Pepper Creek

Water temperature in Pepper Creek generally increased throughout the sampling period in both years, ranging from 16 °C in May to 32 °C in September (Fig. 6). Although variation in salinity was observed on a tidal scale, salinity generally ranged from 15‰ to 25‰ throughout the study period. On occasion, however, salinities <5‰ were observed and occasionally lasted for several days coincident with storm water runoff (Figs. 6*d*, 6*e*, and 6*f*).

Of the physicochemical parameters measured, DO exhibited the greatest variability. Spatial differences in DO were minimal, while dynamic temporal fluctuations in DO were observed over the diel cycle and seasonally, during both

Fig. 4. Mean (±standard error, SE) RNA:DNA for (*a*) summer flounder (*Paralichthys dentatus*) and (*b*) weakfish (*Cynoscion regalis*) in response to changes in feeding status ($n = 5$ per time point). Asterisks (*) indicate the time point at which RNA:DNA significantly differs from fully fed (day 3) or starved (day 8) levels ($p < 0.05$).

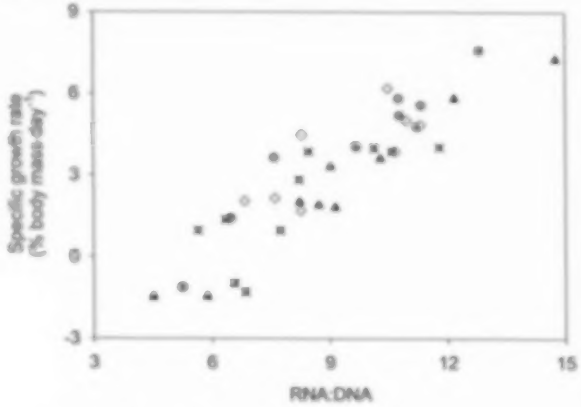


years and at all sites (Fig. 6). Even though DO concentrations in Pepper Creek occasionally varied between 0.01 (essentially anoxia) and 20 mg $O_2 \cdot L^{-1}$ over 24 h, mean DO conditions rarely dropped below the 24 h growth protective DO criteria of 4.8 mg $O_2 \cdot L^{-1}$ and, on only a few occasions, dropped below the 24 h survival protective DO criteria of 2.3 mg $O_2 \cdot L^{-1}$ established by the USEPA (2000). In fact, it is noteworthy that the 24 h moving average DO concentrations were generally at or above temperature- and salinity-dependent saturation concentration (~6.5 to 8.5 mg $O_2 \cdot L^{-1}$) throughout this study (Fig. 6).

Patterns of fish abundance

Juvenile summer flounder were present in trawl collections from early May through July, and juvenile weakfish were present from early July through August. Summer flounder were scarce at Site 3 in 2002, as indicated by the few RNA:DNA observations noted (Fig. 7*c*). Although total abundance and catch per unit effort were not quantified in this study, we observed no obvious differences in abundance among sites in 2003, when both species were present in greater numbers than in 2002.

Fig. 5. Relationship between RNA:DNA and specific growth rates (SGR) of juvenile weakfish (*Cynoscion regalis*) exposed to normoxia (7 mg $O_2 \cdot L^{-1}$, triangles) and three levels of hypoxia (2 mg $O_2 \cdot L^{-1}$, circles; 3.5 mg $O_2 \cdot L^{-1}$, diamonds; and 5 mg $O_2 \cdot L^{-1}$, crosses) at 25 °C.



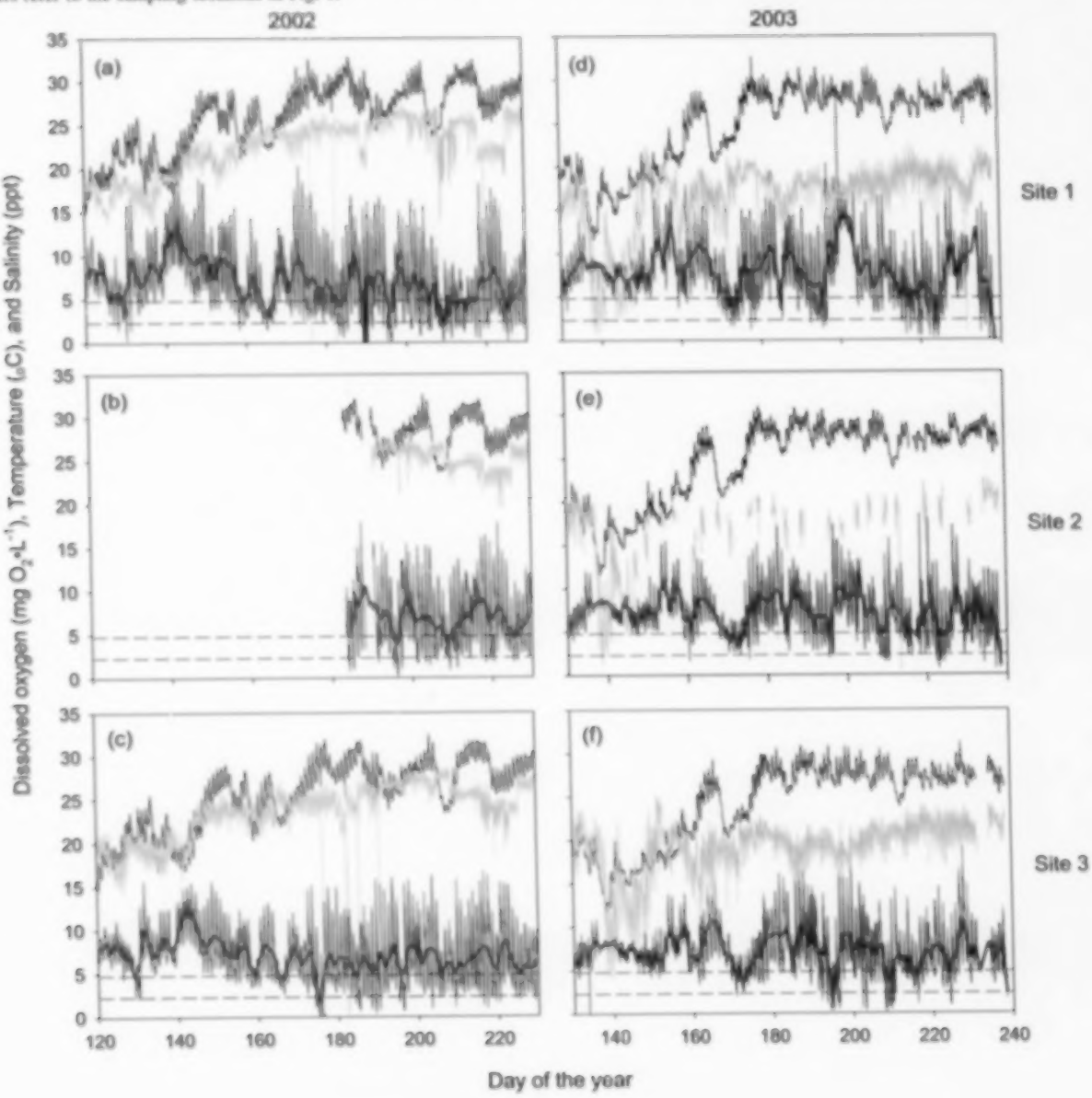
In situ condition indices and DO

In 2002 and 2003, RNA:DNA and stomach content mass were measured in juvenile summer flounder ($n = 180$, 43–104 mm SL) and weakfish ($n = 200$, 54–85 mm SL) collected from Pepper Creek (Table 3). RNA:DNA of both species were remarkably dynamic over the two sampling years and often varied greatly over the 3- to 4-day sampling intervals (Fig. 7, all). Plots of RNA:DNA versus DO suggested that physiological condition in these two species vary temporally in response to changes in prevailing DO conditions (Fig. 7).

The canonical correlation analyses provide a canonical variable (referred to here as condition) that can be regarded as a composite of RNA:DNA and stomach content mass. Examination of the coefficients used to calculate the condition variate from RNA:DNA and stomach content mass, and the correlations between the condition canonical variate and RNA:DNA and stomach content mass, showed that the canonical condition variate was strongly dominated by RNA:DNA. For example, correlations between the condition variate and RNA:DNA had median values of 0.97 in weakfish and 0.95 in summer flounder. In contrast, the median correlations between the condition variate and stomach content mass were 0.26 in weakfish and 0.13 in summer flounder. Additionally, the correlations between RNA:DNA and stomach content mass ranged from 0.06 to 0.11 in weakfish and –0.22 to –0.17 in summer flounder. A scatterplot of the observed stomach content mass vs. RNA:DNA (not shown) also showed no apparent relationship between the two variates.

Both weakfish and summer flounder consistently showed positive correlations between RNA:DNA and mean oxygen values at all time spans (Figs. 8*a*, 8*b*). Stomach content mass was more weakly correlated with mean oxygen values in both species at long time spans (Figs. 8*a*, 8*b*). At shorter time spans (i.e., less than 10 h), weakfish stomach content mass (Fig. 8*b*) was negatively correlated with mean dissolved oxygen, and summer flounder correlations (Fig. 8*a*) varied about zero.

Fig. 6. Temperature (°C; blue, upper line), salinity (‰; orange, middle line), and dissolved oxygen (DO, mg $O_2 \cdot L^{-1}$; gray, bottom line) conditions in Pepper Creek during 2002 and 2003. The solid red line is the 24 h moving average DO. The lower and upper broken lines indicate the USEPA (2000) growth protective (2.3 mg $O_2 \cdot L^{-1}$) and survival protective (4.8 mg $O_2 \cdot L^{-1}$) DO criteria, respectively. Site numbers refer to the sampling locations in Fig. 1.



Correlations of RNA:DNA and stomach content mass of summer flounder with DO_{CV} (DO variability) were weakly positive and negative, respectively (Fig. 8*c*). Correlations of both RNA:DNA and stomach content mass were weakly negative for weakfish (Fig. 8*d*). Correlations between stomach content mass and DO_{CV} showed a similar pattern for both species, with higher positive correlations only occurring shortly before the fish were collected.

Finally, juvenile summer flounder and weakfish appear to be more negatively impacted by hypoxia in the wild than would be expected based on existing laboratory data. In nearly all instances, in situ SGR estimates of both species were substantially lower than or equal to growth rates predicted by the laboratory growth models (Fig. 9). In those cases where in situ SGR exceeded SGR_{pred}, DO conditions were generally normoxic (~7 mg $O_2 \cdot L^{-1}$) to slightly supersa-

Fig. 7. Relationship between RNA:DNA of juvenile summer flounder (*Paralichthys dentatus*, triangles) and weakfish (*Cynoscion regalis*, circles) and dissolved oxygen (DO, mg $O_2 \cdot L^{-1}$) in Pepper Creek during 2002 and 2003. The gray line represents all DO observations, and the black line is the 24 h moving average DO. Site numbers refer to the sampling locations in Fig. 1.

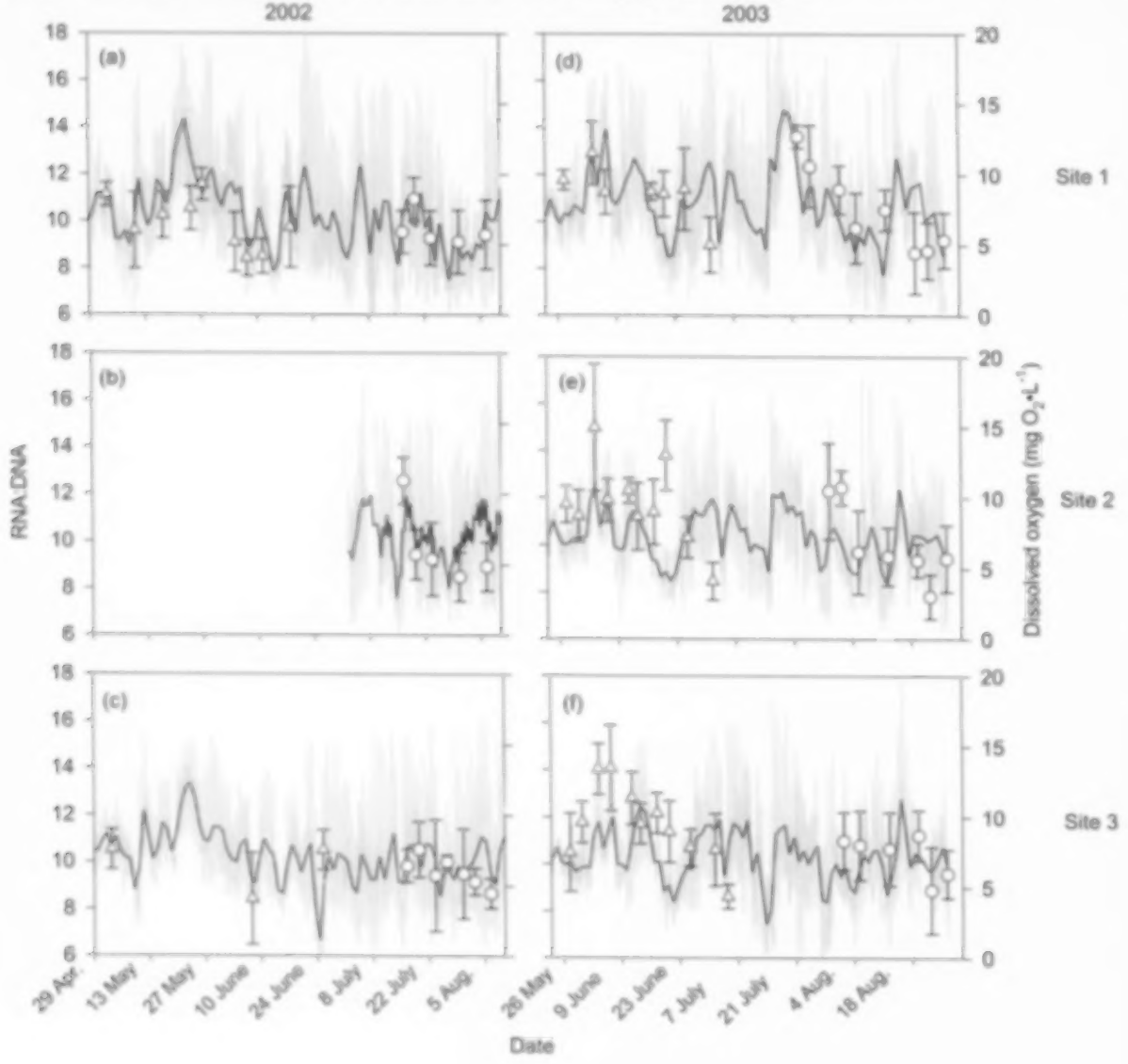
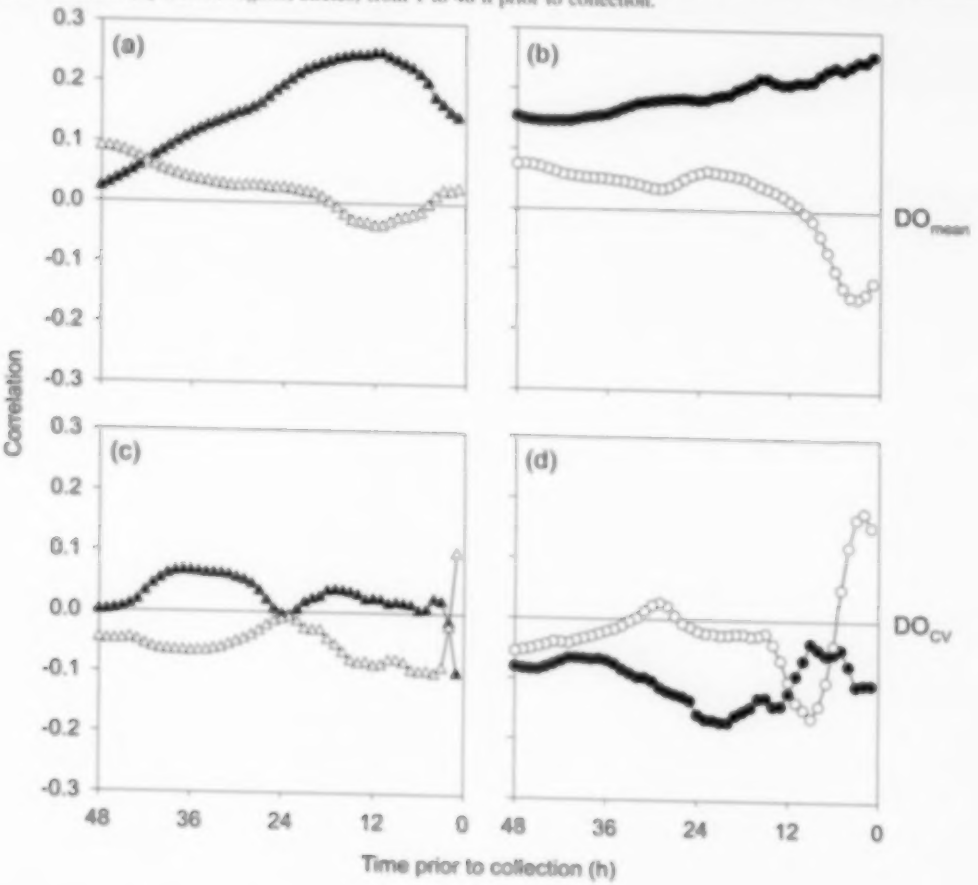


Table 3. Summary of RNA:DNA and stomach content mass (g) for juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) collected from Pepper Creek.

	RNA:DNA			Stomach content mass		
	Mean (SD)	Min.	Max.	Mean (SD)	Min.	Max.
Summer flounder	10.99 (1.99)	6.48	19.46	0.21 (0.18)	0.02	1.07
Weakfish	9.97 (1.84)	6.35	20.63	0.17 (0.21)	0	2.20

Fig. 8. Correlograms showing the correlation among condition indices (RNA:DNA, solid symbols; stomach content mass, open symbols), mean dissolved oxygen concentration (DO_{mean}), and DO variability (DO_{CV}) for (*a* and *c*) summer flounder (*Paralichthys dentatus*, triangles) and for (*b* and *d*) weakfish (*Cynoscion regalis*, circles) from 1 to 48 h prior to collection.



turated (>7 mg $O_2 \cdot L^{-1}$), although not all periods of supersaturation produced in situ growth rates comparable with predicted values. In addition, a pattern of greater proportional reduction in in situ growth rates (relative to predicted rates) with decreasing DO is more apparent for summer flounder than for weakfish (Fig. 9).

Discussion

Since a large percentage of fishery species relies on estuaries and other shallow coastal habitats as nurseries, the continued decline of water quality in these environments is of particular concern. If hypoxia alters habitat use or physiological fitness, it could substantially reduce the quantity and quality of these critically important habitats. Gibson (1994) emphasized the importance of "natural experiments" to identify the effects of environmental factors on habitat quality and (or) quantity and their anticipated effects on recruitment and production of fishes. To our knowledge, this is the first study to provide direct evidence of a functional relationship between DO and growth rates of free-ranging fishes in estuarine or marine systems.

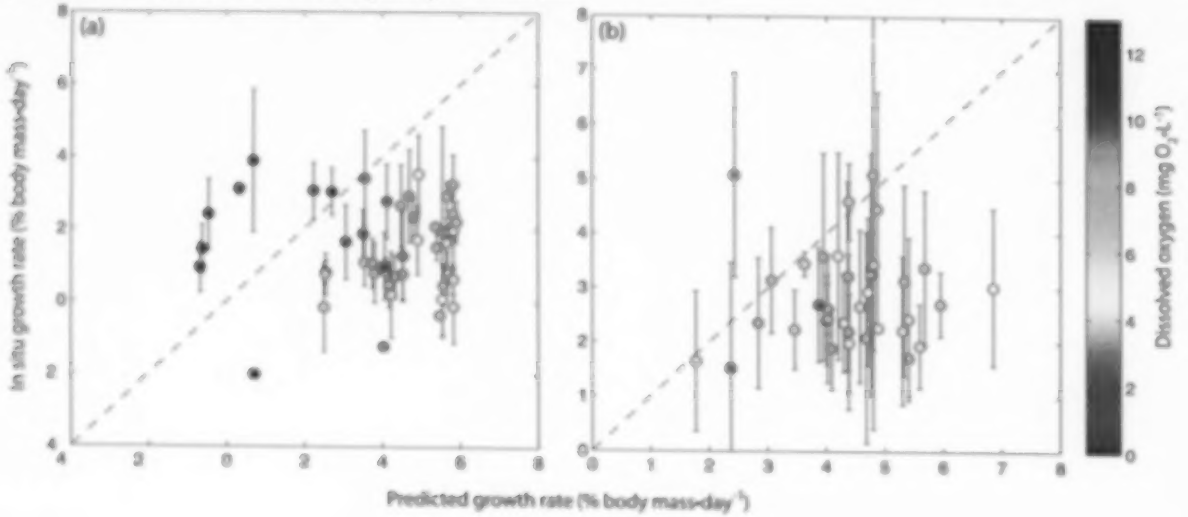
Relationship between RNA:DNA and growth

Collectively, the results of these laboratory studies clearly demonstrate the utility of RNA:DNA as a proxy for growth rate in free-ranging juvenile fishes in field studies, particularly in those environments where physicochemical conditions vary over relatively small temporal (e.g., hours to days) and spatial scales.

First, SGR was strongly and positively related to RNA:DNA in both summer flounder and weakfish. As in previous studies, temperature had a significant effect on this relationship (Buckley et al. 1984; Malloy and Targett 1994; Peck et al. 2003). Although body mass had a significant effect on this relationship for weakfish, this was not the case for summer flounder in this study or in a previous study by Malloy and Targett (1994). Therefore, the influence of body mass on the relationship between RNA:DNA and SGR will likely need to be considered independently on a per-species basis.

Furthermore, the RNA:DNA of juvenile summer flounder and weakfish responded quickly to changes in ration and, presumably, growth rate. RNA:DNA rapidly decreased fol-

Fig. 9. Relationship among dissolved oxygen (DO), in situ specific growth rate (SGR, mean ± standard deviation (SD) estimated by RNA:DNA), and predicted SGR (SGR_{pred} based on experimental data) of juvenile (*a*) summer flounder (*Paralichthys dentatus*) and (*b*) weakfish (*Cynoscion regalis*) in Pepper Creek. The broken line indicates the one-to-one relationship. The color bar indicates the average DO concentration (mg $O_2 \cdot L^{-1}$) for the 24 h period prior to collection.



lowing the removal of food, and in a similar fashion, rapidly increased upon refeeding. These results are consistent with the earlier work on summer flounder (Malloy and Targett 1994), but to our knowledge are the first to describe the response of RNA:DNA at less than 24 h resolution. Because of the experimental design, we were unable to determine whether RNA:DNA achieved a constant minimum starvation level or ultimately exceeded fully fed initial levels following refeeding.

Finally, the relationship between RNA:DNA and SGR in juvenile weakfish was independent of ambient DO concentrations. While numerous studies describe the relationship between RNA:DNA and growth rate in a variety of fish taxa, few have examined the potential influence of hypoxia on the decoupling of this relationship (see Zhou et al. 2001). Therefore, it is assumed that the observed changes in RNA:DNA relative to DO conditions in the field portion of this study are indicative of changes in feeding status and (or) growth rate and are not due to other physiological responses to oxygen stress (e.g., the upregulation of stress enzymes or production of antioxidants) that are not directly involved in the growth process.

DO and fish condition in Pepper Creek

The severity of hypoxia in Pepper Creek during this study could be described as moderate according to current water quality standards based on mean DO concentrations (USEPA 2000). Hypoxia is often defined as DO concentrations below 2 mg $O_2 \cdot L^{-1}$ — levels generally considered to be stressful to estuarine fauna (Diaz and Rosenberg 1995, 2008; Diaz 2001). Although DO in Pepper Creek exhibited a characteristic diel cycle, there was also longer-term variability in average DO concentration and diel variability (difference between daily minimum and maximum DO) on the order of several days to weeks. Mean DO conditions were generally above 5 mg $O_2 \cdot L^{-1}$ and rarely dropped below the USEPA (2000) growth protective criteria of 4.8 mg $O_2 \cdot L^{-1}$.

RNA:DNA and stomach content mass were included in the canonical correlation analysis in an effort to differentiate between a direct effect of DO and a possible indirect effect of reduced feeding and prey availability on the RNA:DNA ratio. It appears that recent prey consumption and RNA:DNA are not strongly related. This is to be expected, as there will be some degree of lag between the time of food ingestion and the eventual increase in RNA and protein production, as shown in this laboratory portion of this study as well as others.

However, the correlations between previous mean DO values and RNA:DNA were consistently positive, albeit small, for both species. These correlations are on a time scale similar to that necessary to observe a significant increase or decrease in growth rate in this laboratory study (i.e., 18–48 h). Stomach content mass was also weakly and positively correlated with mean DO concentrations at longer time scales. At short time scales (<12 h), however, the correlations between stomach content mass and mean DO became negative in weakfish, but remained close to zero in summer flounder. This negative correlation (i.e., decreased feeding at high DO concentrations) at short time scales in weakfish may result from a lag in the onset of feeding as DO concentrations rapidly increase throughout the day. It is tempting to speculate that summer flounder, as a benthic and more sedentary fish, do not show a similar pattern.

Correlations among RNA:DNA, stomach content mass, and DO_{CV} show a largely indeterminate pattern, although most of the correlations are negative, as would be expected — it is easy to surmise that high variability in DO concentration would suppress feeding and RNA production.

These results provide unique insight into the potential impact of relatively moderate diel cycling hypoxia on the

growth of juvenile fishes in the wild. Prior caging studies have observed differences in growth rate between habitats and attributed those differences to differences in DO conditions along an anthropogenic gradient (Meng et al. 2001) and with depth (Eby et al. 2005). However, the degree to which these studies represent growth rates of free-ranging individuals is uncertain, given the potential for accumulation of prey (Meng et al. 2000), decreased energetic costs of foraging and avoiding predators (Meng et al. 2000), and restricted movements of fishes that might otherwise avoid hypoxic environments (Meng et al. 2001). Moreover, the necessarily long time periods over which such caging studies integrate growth make the inference of causal relationships between environmental conditions and growth rate difficult. This study describes dynamic physiological responses of free-ranging fishes in an estuarine system that undergoes short-term variability in DO.

Furthermore, growth rates observed in summer flounder and weakfish from Pepper Creek were consistently and substantially lower compared with growth rates predicted from existing laboratory data. In the laboratory, moderate yet significant reductions in the growth rate of summer flounder occurred only after 7 days at chronic DO ≤3.5 mg $O_2 \cdot L^{-1}$ (at 20 and 25 °C) (Stierhoff et al. 2006) or under diel cycling conditions (2–11 mg $O_2 \cdot L^{-1}$) at temperatures ≥25 °C (Stierhoff et al. 2006). In contrast, growth rates of weakfish in the laboratory were unaffected at constant DO as low as 2 mg $O_2 \cdot L^{-1}$ and under diel cycling DO (2–11 mg $O_2 \cdot L^{-1}$) for 7 days (Stierhoff et al. 2009). While it might be expected that in situ growth rates would be lower than those observed under ad libitum feeding conditions in laboratory studies (because of variability in prey resources, energy expended during predator avoidance, etc. in the wild), the low in situ growth rates observed throughout this study were somewhat unexpected given the infrequent occurrence of severe hypoxia during either year. While some caution in these comparisons is warranted given the weak relationship between DO and growth rates of weakfish in the laboratory (adjusted $R^2 = 0.16$), these comparisons of laboratory and in situ growth rates suggest that estuary-dependent fishes may experience growth limitation at DO concentrations higher than those considered harmful under the existing laboratory-based criteria.

Although growth rate is undoubtedly an important factor that influences the survival of larval and juvenile fishes, trade-offs may exist whereby habitats that are suboptimal for growth may benefit survival by reducing predation pressure and (or) competition (Pihl et al. 1991; Eby and Crowder 2002). Such trade-offs between growth rate and survival make the description of essential fish habitat increasingly difficult and place further emphasis on the need for growth rate and mortality estimates from a variety of habitats to better identify those habitats that serve as nurseries.

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Influence of diel cycles of respiration, chlorophyll, and photosynthetic parameters on the summer metabolic balance of temperate lakes and rivers

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Abstract: Primary production, planktonic respiration, bacterial abundance, and chlorophyll *a* were measured in the epilimnion of two Canadian Shield lakes and in two large rivers to establish their metabolic balance and to contrast oligotrophic and oligo-mesotrophic systems. Pronounced diel respiration cycles were observed in all systems, with a minimum in the morning and a maximum in the evening. Respiration was positively correlated with water temperature, incident light, and chlorophyll *a* concentration. Diel cycles of chlorophyll *a* were discernible in both rivers and in the oligo-mesotrophic lake. Our results show that a single morning sampling underestimates both respiration and chlorophyll *a*, whereas a single sample taken at noon generally agrees better with the average of four daily measurements. In oligotrophic Lac Croche, the photosynthetic parameter α^B remained constant throughout the day, whereas P_m^B increased by 12.5% between 0600 h and 1300 h. Ignoring the diel community respiration cycle resulted in a ~25% underestimation of daily respiration, whereas ignoring the P_m^B cycle induced a ~9% underestimation of the daily primary production. The net balance between production (*P*) and respiration (*R*) of the oligotrophic lake epilimnion remained positive during two summers, and the *P*:*R* ratio showed net autotrophy for all systems except for the oligotrophic river, which was slightly net heterotrophic.

Résumé : La production primaire, la respiration planctonique, l'abondance bactérienne et la chlorophylle *a* ont été mesurées dans l'épilimnion de deux lacs du Bouclier canadien et dans deux grandes rivières afin d'établir leur bilan métabolique et de comparer des milieux oligotrophes et oligo-mésotrophes. Des cycles journaliers de respiration ont été observés dans les quatre milieux aquatiques avec un minimum en matinée et un maximum en soirée. La respiration était corrélée positivement avec la température de l'eau, la lumière incidente et la concentration en chlorophylle *a*. Des cycles journaliers de la chlorophylle *a* ont été mis en lumière dans les deux systèmes fluviaux et dans le lac oligo-mésotrophe. Le prélèvement d'un seul échantillon en matinée sous-estime la respiration et la chlorophylle *a*, tandis qu'un seul prélèvement effectué à midi correspond généralement mieux à la moyenne de quatre mesures journalières. Dans le lac Croche qui est oligotrophe, le paramètre photosynthétique α^B ne présentait pas de variation journalière, alors que P_m^B augmentait de 12,5 % entre 0600 h et 1300 h. Ignorer la variation journalière de la respiration engendre une sous-estimation de la respiration journalière de ~25 %, tandis que l'omission du cycle de P_m^B produit une sous-estimation ~9 % de la production primaire journalière. Le bilan net entre la production (*P*) et la respiration (*R*) de l'épilimnion du lac oligotrophe est demeuré positif au cours de deux étés de mesure et le rapport *P*:*R* indique que tous les systèmes étaient autotrophes nets à l'exception de la rivière oligotrophe, faiblement hétérotrophe.

Introduction

The importance of allochthonous organic carbon as a source of energy in oligotrophic systems has been a subject of debate for many years. Some studies suggest that planktonic respiration commonly exceeds primary production in the epilimnion of oligotrophic lakes and in the photic zone of rivers (del Giorgio and Peters 1994; Coveney and Wetzel 1995; del Giorgio et al. 1997*a*), whereas others have concluded that the epilimnion of oligotrophic lakes and oceans are net autotrophic (Williams 1998; Carignan et al. 2000; Berman et al. 2004). Russ and collaborators (2004) found that an oligotrophic lake alternated between net autotrophy and heterotrophy according to the stratification conditions of the water column (Russ et al. 2004).

Most studies have measured daily production and respiration from samples collected at a single set time without considering the possible influence of diel cycles in planktonic respiration and photosynthetic parameters on the estimation of metabolic balances. Diel respiration cycles are well documented in systems ranging from mesotrophic to eutrophic (Markager et al. 1992; Szyper et al. 1992; Teichert-Coddington and Green 1993), but have never been reported for oligotrophic environments because of the lack of precision of oxygen methods. Diel cycles of bacterial abundance (Fuhrman et al. 1985) and metabolism have, however, been reported on a few occasions (Straskrabova and Fuksa 1982; Chin-Leo and Benner 1991; Simon 1994).

Marine phytoplankton biomass, estimated as chlorophyll *a*, has also been shown to follow diel cycles, with a maxi-

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Table 1. Seasonal mean values of physical and chemical characteristics of the four aquatic systems of the epilimnion for lakes and the water column for rivers.

	T (°C)	K (m^{-1})	Z_{eps} (m)	Z_m (m)	TP ($mg \cdot m^{-3}$)	DOC ($g \cdot m^{-3}$)	Chl-a ($mg \cdot m^{-3}$)	Bact. (10^6 cells·mL^{-1})
Lac Croche (1998)	21.2	0.64	2.7	5.1†	3.8†	3.5†	1.4	—
Lac Croche (2000)	19.4	0.67	2.7	5.1†	3.8†	3.5†	1.1	0.75
Lac à l'Ours (2000)	18.0	1.63	2.0	6.7†	14.0†	6.6†	4.0	2.40
St. Lawrence River (2000)	22.1	0.37	8.0*	8.0*	10.0	2.2	1.0	6.51
Ottawa River (2000)	22.0	2.09	4.8*	4.8*	44.0	6.9	2.1	4.40

Note: T, water temperature; K, light attenuation coefficient; Z_{eps}, epilimnetic depth or mean river depth (based on Blais 2000); Z_m, mean depth (based on Blais 2000); TP, total phosphorus concentration; DOC, dissolved organic carbon concentration; Chl-a, chlorophyll a concentration; Bact., bacteria abundance.

*Data from Blais 2000.

†Data from Carignan et al. 2000.

mum during daytime and a minimum at night (Sournia 1975; Le Bouteiller and Herbland 1982; Fuhrman et al. 1985). To our knowledge, such cycles have never been reported in lakes and rivers. Here, we show that diel respiration and chlorophyll cycles in lakes and rivers do occur and that these in turn influence the outcome of metabolic balance estimates.

Although some authors have detected diel cycles in photosynthetic parameters (α^B, the initial slope of the photosynthetic-light response curve, and P_m^B, the light-saturated photosynthesis rate) in mesotrophic and eutrophic systems (MacCaull and Platt 1977; Harding et al. 1982*a*; Vandevelde et al. 1989), these cycles have been identified in oligotrophic freshwater systems exclusively from ^{14}C incubations. This technique is known to underestimate gross primary production (GPP) (Peterson 1980) and could therefore lead to inaccurate estimates of the production–respiration (P–R) balance, especially in oligotrophic waters characterized by low rates of production and respiration. To palliate to this methodological problem, various oxygen-based methods have been proposed to study P–R balance in aquatic systems, such as the high-precision Winkler method (Carignan et al. 1998), the use of ^{18}O-labelled water (Emerson et al. 1991; Russ et al. 2004; Ostrom et al. 2005), and the deployment of oxygen sondes (Coloso et al. 2008).

Using a high-precision oxygen method, we examined the presence of diel periodicity in community respiration and phytoplankton biomass in the epilimnion of two Canadian Shield lakes and in the water column of two large rivers of different trophic status. The influence of such variations on the summertime metabolic balance of these systems is estimated.

Materials and methods

Study site

Respiration and photosynthetic parameters were measured in two contrasting Shield lakes and two large rivers near Montréal (Quebec, Canada). Lac Croche and Lac à l'Ours are two small lakes (areas of 15 and 20 ha, respectively) located approximately 80 km northwest of Montréal (Lac Croche: 45°59′N, 74°01′W; Lac à l'Ours: 45°58′N, 74°04′W). Lac Croche is oligotrophic, whereas the watershed of Lac à l'Ours drains a 22 ha wetland, which imparts mesotrophic characteristics to the lake (Table 1). Submerged aquatic vegetation is virtually absent from Lac Croche and covers less than 1% of Lac à l'Ours. Both lakes are further described in Carignan et al. (2000).

The two rivers show strongly contrasting physical and chemical characteristics (Table 1). The St. Lawrence River, sampled downstream from Lake St. Francois (45°15′N, 74°09′W), is characterized by transparent and oligotrophic waters. The Ottawa River, which drains Canadian Shield forests and agricultural lowlands, was sampled at the outlet of Lac des Deux-Montagnes, before its confluence with the St. Lawrence River (45°24′N, 74°00′W); it is characterized by brown-coloured waters moderately affected by agriculture and urban sewage.

Sampling

Diel respiration, chlorophyll a, and bacterial abundance cycles were measured every 6 h in 1998 in Lac Croche only and at all study sites in 2000 during periods of 24–48 h between early June and late August for a total of four periods in each lake and three in each river. Photosynthesis vs. irradiance (E, mol photons·m^{-2}·s^{-1}) curves and derived photosynthetic parameters α^B (the initial slope of the photosynthetic-light response curve) and P_m^B (the light-saturated photosynthesis rate) were determined in Lac Croche four times in the morning (0600 h) and again in the evening (1800 h) in 1998 and three times in the morning (0600 h) and the afternoon (1300 h) in 2000. During these two summers, additional measurements were conducted to calculate the seasonal P–R balance of the epilimnion.

In the lakes, depth-integrated samples were taken between the surface and the lower limit of the epilimnion, defined as the depth at which the temperature gradient became larger than 1 °C·m^{-1}. In the rivers, samples were taken just below the surface using a Van Dorn bottle because these systems were well mixed. Water was poured into opaque 20 L polyethylene carboys and transported to the laboratory for metabolic and biomass measurements within 1 h in insulated containers to minimize temperature change. Photosynthetically active radiation (PAR) and ultraviolet-B (UVB) were recorded every 15 min at the nearby Station de Biologie des Laurentides (1998 and 2000), whereas PAR only was recorded on the roof of the St. Lawrence Centre building in Montréal (2000).

Metabolic measurements

At the laboratory, collected water used for metabolic rate measurements was dispensed into 15 clear and 4 dark 300 mL Pyrex bottles, allowing for a 900 mL overflow. The bottles were placed in a light gradient incubator similar to the one described in Shearer et al. (1985), equipped with a Phillips MH1000, 1000 W metal-halide lamp. It provided five irradiance levels, with triplicate clear bottles at each light level ranging from ~30 to ~1100 $\mu E \cdot m^{-2} \cdot s^{-1}$, as measured at several points inside replicate bottles with a Biospherical QSL-101 4-π quantum meter. Incubations were performed within 1 °C of the temperature measured in situ and lasted 4 h for production measurements and 4–6 h for respiration measurements. A high-precision (±2 $\mu g \cdot L^{-1}$, standard error (SE) of triplicate determination) Winkler method was used to determine the net primary production of oxygen (NPP) and community respiration (R) from changes in dissolved oxygen in clear and dark bottles during the incubation (see Carignan et al. 1998 for details). Briefly, dissolved O_2 was fixed by injecting 2.0 mL of $MnCl_2$ in each biological oxygen demand (BOD) bottle, followed by 2.0 mL of alkaline iodide. Bottles were immediately stoppered and shaken vigorously. After settlement of flocculent material, 2.2 mL of H_2SO_4 were added, and bottles were once again shaken vigorously. The titration was performed in dynamic equivalence detection mode using a Metrohm DMS titrator equipped with a 10 mL burette, a Metrohm Pt titrode, and a standard solution of thiosulfate. The O_2 concentration was calculated using eq. 2 of appendix 1 in Carignan et al. (1998). GPP was calculated as the sum of NPP and R.

Photosynthetic parameters were determined using the PSPARMS program (version 4.0, Fee 1990) based on the semi-empirical model presented in Jassby and Platt (1976). Daily epilimnetic GPP was calculated using DPHOTO (version 4.0, Fee 1990). YPHOTO (version 4.0, Fee 1990) was used to integrate GPP for the entire summer in Lac Croche from daily incident irradiance, chlorophyll a concentrations, and epilimnetic depths.

Chlorophyll *a* measurements

Triplicate water samples (500 to 1000 mL) were passed through Whatman GF/C filters, which were frozen at –30 °C until analysis. Pigments were extracted for 24 h in 12 mL of 90% ethanol and read at 665 and 750 nm, before and after acidification, on a Spectronic 301 spectrophotometer (Sartory and Grobbelaar 1984).

Bacterial abundance measurements

During the diel variation experiments, triplicate subsamples were collected for bacterial counts, were stored immediately at 4 °C, and were analysed within 24 h of collection. Samples were analysed with a FACScan (Becton Dickinson) flow cytometer after coloration with the green nucleic acid stain SYTO-13 (final concentration of 2.5 $\mu mol \cdot L^{-1}$) and calibration beads (del Giorgio et al. 1996).

Data analysis

To determine the presence of diel cycles in respiration and environmental variables, the data were standardized ($(x - \mu)/\sigma$), where x is the variable measured over the diel cycle, μ is the average of the data obtained over a 24 h (i.e., from 0600 the same day to 0600 the following day) period, and σ is the standard deviation of that period. The standardization of the data allows the study of diel cycle by excluding the effects of seasonal variability, where for example respiration will be higher when the water temperature is higher. One-way and repeated measures analyses of variance (ANOVA) were used to determine the occurrence of significant differences between the means obtained at different times of the day (0600, 1200, 1800, 2400, and 0600 the following morning) using quantitative standardized data for all sampling dates. The repeated measures ANOVA is specific to sampling design where measurements are repeated (in this case, at different times of day) for the same subject (in this case, the same lake) over a period of time (over the course of the summer). Student t tests for paired samples were used to detect significant differences between absolute values of paired measurements taken during the diel cycle and to compare photosynthetic parameters measured in the morning and afternoon.

Results

Community respiration ranged from 82 to 288 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ in Lac Croche and from 233 to 697 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ in Lac à l'Ours (Table 2). Respiration ranged between 113 and 311 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ for the St. Lawrence River and between 67 and 444 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ for the Ottawa River. All systems showed important diel cycles (both one-way and repeated measures ANOVA, $p < 0.05$) in community respiration whenever the systems were subjected to high irradiances (Figs. 1 and 2*a*, Table 2).

Respiration rates were lowest in the morning, increased to a maximum during the evening, and decreased again overnight. In Lac à l'Ours, however, the highest values tended to occur at midday. In every case, the differences between the lowest and highest standardized rates were significant (t test, $p < 0.05$). Across all study sites, using absolute values of volumetric respiration to estimate daily respiration from a single morning measurement (0600 h) caused a 17% to 35% underestimation of the average rates obtained from four measurements obtained at 6 h intervals (Table 3). Similarly, estimating chlorophyll concentration from a single morning measurement (0600 h) in the two rivers caused a 19% to 21% underestimation of the average concentrations obtained from three measurements obtained at 6 h intervals (night samples were excluded from this comparison because their use for computation of primary production would have biased the results), whereas no significant underestimation was found in the two lakes (Table 4). The diel respiration cycle was significantly related to PAR, chlorophyll a, and temperature when night measurements were removed from the analyses (Table 5). However diel temperature variations were too small (1 to 2 °C, Table 2) to explain the 50% to 100% changes in respiration.

Respiration rates and chlorophyll a concentrations followed similar patterns (Figs. 2*a* and 2*b*), with significant increases of the absolute values of 50% to 100% during daytime (paired t test for each aquatic system, $p < 0.05$, t = 2.64 to 5.20, number of observations (n): 6 or 8, and degrees

Table 2. Average diel minimum (min.) and maximum (max.) ± standard deviation values of variables observed at 6 h intervals during 24 or 48 h periods in 1998 and 2000.

		Temperature (°C)		PAR (mol photons·m^{-2}·day^{-1})		Respiration (mg O_2·m^{-3}·day^{-1})		Bacterial abundance (10^6 cells·mL^{-1})		Chlorophyll *a* (mg·m^{-3})	
Aquatic system	*n*	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Lac Croche 1998	6	22.0±1.1	24.4±0.9	40.7±14.9	46.9±9.2	130±50	208±43	—	—	1.2±0.0	1.5±0.5
Lac Croche 2000	8	19.3±3.3	21.0±3.4	17.7±10.1	39.4±20.2	118±12	223±44	0.60±0.14	0.81±0.09	0.9±0.3	1.3±0.4
Lac à l'Ours	8	19.4±3.2	21.3±3.3	17.7±10.1	39.4±20.2	299±52	582±84	1.67±0.72	2.39±0.73	4.3±3.1	9.2±5.3
St. Lawrence River	6	20.3±1.3	21.8±1.0	32.1±13.1	49.0±4.8	134±30	280±32	5.10±1.27	9.17±5.20	0.8±0.2	1.4±0.1
Ottawa River	6	21.5±1.6	22.9±1.4	32.1±13.1	49.0±4.8	121±60	302±126	3.59±1.60	5.13±2.57	1.8±0.4	3.2±1.0

Note: The number of 24 h sampling periods (*n*) is also shown. PAR, photosynthetically available radiation.

Table 3. Mean ± standard deviation values of daily planktonic respiration (mg O_2·m^{-3}·day^{-1}) estimated from different times of sampling and different combinations of samples in Lac Croche (n = 14), Lac à l'Ours (n = 8), St. Lawrence River (n = 6), and Ottawa River (n = 6).

					Average	
Aquatic system	0600	1200	1800	2400	0600 and 1800	0600, 1200, 1800, and 2400
Lac Croche	139±38 (1.17)*	157±29 (1.04)	173±44 (0.94)	181±49 (0.90)	156±39 (1.04)	163±29
Lac à l'Ours	368±64 (1.24)*	534±104 (0.85)*	467±111 (0.98)	455±121 (1.00)	418±58 (1.09)*	456±76
St. Lawrence River	176±39 (1.23)	206±47 (1.05)	247±42 (0.87)	234±42 (0.92)*	212±24 (1.02)	216±16
Ottawa River	150±77 (1.35)*	156±50 (1.30)*	258±119 (0.79)*	248±85 (0.82)*	204±94 (1.00)*	203±80

Note: The ratios between the estimated respiration from a diel cycle and the measured values are presented in parentheses. The significant differences from a paired *t* test ($p < 0.05$) between measured respiration and estimated values using a diel cycle are identified with an asterisk (*).

Table 4. Mean ± standard deviation values of daily chlorophyll *a* concentration (mg·m^{-3}) estimated from different times of sampling and different combinations of samples in Lac Croche (1998 and 2000, n = 14), Lac à l'Ours (n = 8), St. Lawrence River (n = 6), and Ottawa River (n = 6).

					Average	
Aquatic system	0600	1200	1800	2400	0600 and 1800	0600, 1200, and 1800
Lac Croche	1.21±0.37 (0.98)	1.20±0.34 (0.99)	1.17±0.28 (1.02)	1.07±0.26 (1.11)*	1.19±0.29 (1.00)	1.19±0.30
Lac à l'Ours	6.25±4.15 (1.09)	8.17±4.42 (0.84)*	6.11±3.13 (1.12)	4.55±2.75 (1.50)*	6.18±3.53 (1.11)*	6.84±3.78
St. Lawrence River	0.93±0.20 (1.19)*	1.07±0.11 (1.04)	1.32±0.11 (0.84)*	0.96±0.07 (1.16)*	1.13±0.12 (0.98)	1.11±0.11
Ottawa River	2.07±0.70 (1.21)*	2.47±0.63 (1.02)	2.99±0.93 (0.84)*	2.25±0.93 (1.12)*	2.53±0.79 (0.99)	2.51±0.72

Note: The ratios between the chlorophyll estimated from a diel cycle and the measured values are presented in parentheses. The significant differences from a paired *t* test ($p < 0.05$) between measured chlorophyll and estimated values using a diel cycle are identified with an asterisk (*).

Fig. 1. Examples of diel variation of (*a*) community respiration and photosynthetically available radiation (PAR, line with shaded area), (*b*) chlorophyll *a*, and (*c*) bacterial abundance in two Laurentian Shield lakes (left) and in two large rivers (right). Lac Croche (open circles) and Lac à l'Ours (solid circles) were sampled on 5–6 June 2000. St. Lawrence River (open circles) and Ottawa River (solid circles) were sampled on 27–28 July 2000.

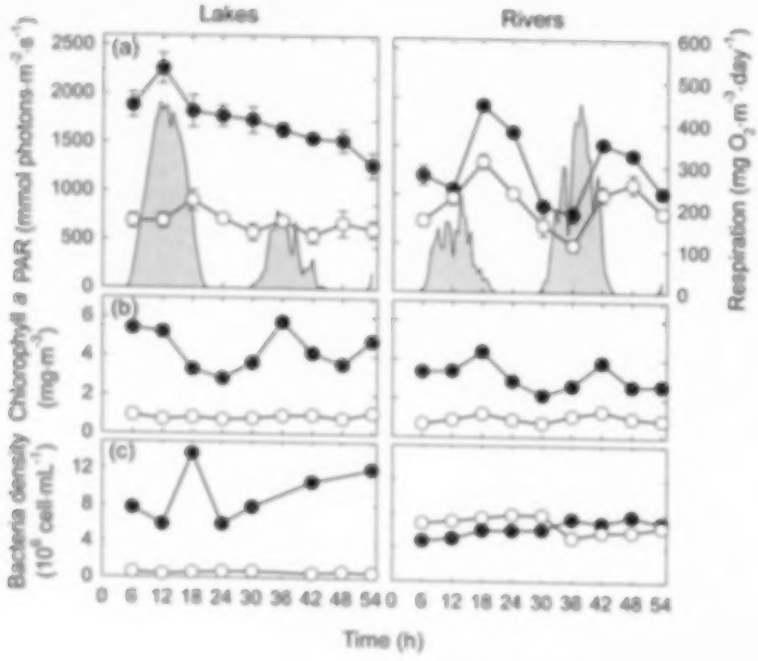


Fig. 2. Seasonal averaged diel cycle of standardized ((value – daily mean)/standard deviation) (*a*) respiration and (*b*) chlorophyll *a* concentration in Lac Croche (1998: $n = 6$; and 2000: $n = 8$), Lac à l'Ours ($n = 8$), Ottawa River ($n = 6$), and St. Lawrence River ($n = 6$) (2000 only for the latter three sites). Each point represents the average (±standard error) of the standardized respiration estimations obtained for each 24 h period. The shaded area indicates the average duration of the night period.

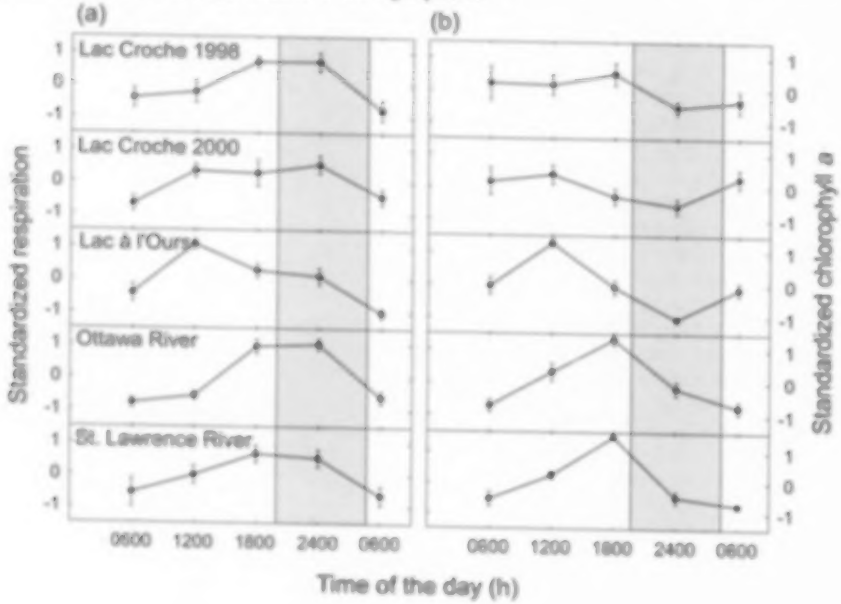


Table 5. Multiple linear regressions relating volumetric respiration (R, mg $O_2 \cdot m^{-3} \cdot day^{-1}$) to water temperature ($T$, °C), mean photosynthetically available radiation in the 6 h preceding sampling (E, mol photons $\cdot m^{-2} \cdot day^{-1}$), and the concentration of chlorophyll a (B, mg $\cdot m^{-3}$).

Aquatic systems	Regression	Adj. R^2	n	p
1,2,3,4	$-0.23\pm0.06 + 0.58\pm0.07\ E$	0.36	136	<0.001
1	$-0.23\pm0.10 + 0.51\pm0.10\ E$	0.29	56	<0.001
1	$-0.17\pm0.10 + 0.45\pm0.10\ T$	0.25	56	<0.001
2,3,4	$-0.23\pm0.08 + 0.63\pm0.08\ E$	0.41	80	<0.001
2,3,4	$-0.22\pm0.08 + 0.63\pm0.09\ B$	0.39	80	<0.001
2,3,4	$-0.09\pm0.08^{*} + 0.56\pm0.08\ T$	0.36	80	<0.001
2,3,4	$-0.17\pm0.07 + 0.44\pm0.09\ E + 0.35\pm0.09\ T$	0.49	80	<0.001

Note: Bacterial abundance (10^6 cells $\cdot mL^{-1}$) was also used in multiple regressions but no significant ($p < 0.05$) relationship was revealed. All variables were standardized ((value – daily mean)/standard deviation), where the average and standard deviation were obtained from data over a 24 h period. Midnight values were not included in the analyses. Aquatic systems used in regression analysis are as follows: 1, Lac Croche; 2, Lac à l'Ours; 3, Ottawa River; 4, St. Lawrence River.

*Intercept not significantly different from 0.

of freedom (df) 5 or 7), except for chlorophyll a concentration in Lac Croche, where the daytime increment was not significant (both ANOVA and paired t test, $p > 0.05$) and where the increase in chlorophyll a amounted to only 9% and 17% in 1998 and 2000, respectively. Total bacterial abundance (living and dead cells), in contrast, showed neither a consistent diel pattern (Fig. 1) nor any relationship with planktonic respiration (Table 5) for any of the aquatic systems.

In Lac Croche, photosynthetic parameters (α^B and P_m^B) estimated at 0600 h and at 1800 h in 1998 were not significantly different (Table 6). In 2000, photosynthetic efficiency (α^B) did not vary significantly between 0600 h and 1300 h. However, the maximum photosynthetic capacity, P_m^B, increased significantly by 12.5% between samples taken at 0600 h and 1300 h (paired t test, $p < 0.05$, t statistic = 4.77, $n = 3$, df = 2). The effect of such an increase in P_m^B on daily GPP in the epilimnion was estimated by calculating GPP for the average values of summer 2000 in Lac Croche of 2.5 m for the epilimnion depth and of 1.1 mg $\cdot m^{-3}$ for chlorophyll a concentration and for PAR irradiance corresponding to that received in mid-July at a latitude of 46°N, with a 34% cloud cover. Estimation of GPP was 9% higher at noon than would be estimated from morning P_m^B values. However, using average P_m^B values (average, standard deviation, n) obtained at 0600 h (12.5, 2.2, 7), 1300 h (14.4, 0.26, 3), and 1800 h (11.6, 2.1, 4) yielded a daily estimation of production only 4% higher than would be obtained using only the morning value of P_m^B.

The seasonal variations of daily primary production and planktonic respiration were determined for the epilimnion of oligotrophic Lac Croche, after accounting for the diel variations in planktonic respiration and chlorophyll concentration (Fig. 3). Over the summers of 1998 and 2000, the average P:R ratio for the epilimnion of Lac Croche was of 1.29, while the net balance (P–R) was +1.24 mmol $O_2 \cdot m^{-3} \cdot day^{-1}$. Daily chlorophyll concentration and volumetric production

Table 6. Average values (standard deviation) of photosynthetic parameters α^B (mg $O_2 \cdot$mg Chl-$a^{-1} \cdot$(mol photons)$^{-1} \cdot m^{-2}$), P_m^B (mg $O_2 \cdot$mg Chl-$a^{-1} \cdot h^{-1}$), and E_k (mol photons $\cdot m^{-2} \cdot s^{-1}$) measured in Lac Croche at 0600 h and 1800 h (summer 1998) and at 0600 h and 1300 h (summer 2000).

Time of day	α^B	P_m^B	E_k
Summer 1998			
0600	22.0±1.3	11.1±1.9	0.50±0.06
1800	27.7±5.9	11.6±2.1	0.44±0.14
Summer 2000			
0600	38.5±6.8	14.4±0.3	0.38±0.07
1300	33.3±4.5	16.2±0.8*	0.50±0.09

Note: The significant differences from a paired t test ($p < 0.05$) between measured parameters at different time of the day are identified with an asterisk (*).

were corrected for the diel cycle in chlorophyll concentration using the ratios estimated in Table 4 and linearly interpolated between times of the day (Fig. 4*b*). The same adjustment was performed on the volumetric respiration using ratios provided in Table 3. Among all four systems, only the St. Lawrence River consistently showed slight net heterotrophic conditions (averaged P:R ratio = 0.68; Figs. 4*a* and 4*b*). When accounting for the diel cycle of chlorophyll and respiration, the Ottawa River samples went from a prevalence of net autotrophic conditions (averaged P:R ratio = 1.15) to a mixture of both net autotrophic and net heterotrophic conditions (averaged P:R ratio = 0.96).

In contrast, the epilimnion of both lakes were only slightly affected by the diel cycle of chlorophyll and respiration. Lac à l'Ours showed mixed conditions (averaged P:R ratio = 0.98), corresponding to results from another mesotrophic lake during the summer season (Ostrom et al. 2005). Lac Croche showed a preponderance of net autotrophic conditions (averaged P:R ratio = 1.29). In all cases, a paired t test showed a significant difference ($p < 0.001$, t statistic = 6.79, $n = 48$, df = 47) between the P:R ratios estimated before and after accounting for the diel cycle of the chlorophyll concentration and respiration.

Discussion

Diel cycle

Our results confirm the occurrence of a diel cycle of planktonic respiration in lakes and rivers, as previously reported in freshwater as well as in marine systems (Markager et al. 1992; Szyper et al. 1992; Teichert-Coddington and Green 1993). Respiration was maximal in the evening and minimal at midnight or in early morning. It is well known that photorespiration contributes substantially to total respiration. Lewitus and Kana (1995) have shown from phytoplankton cultures that light respiration rates can reach up to 13 times the dark respiration rates. However, in a natural plankton population, especially those composed of a majority of heterotrophic organisms, the effect of photorespiration may be lessened (Bender et al. 1987). Moreover, the respiration–light curves presented by Lewitus and Kana (1995) show a decrease in respiration rates at low light levels because of chlororespiration followed by an increase reaching the dark respiration rates at irradiance levels

Fig. 3. Measured epilimnetic gross primary production (GPP, circles) and community respiration (*R*, triangles) and calculated epilimnetic GPP (solid line) of Lac Croche for summer 1998 (*a*) and 2000 (*b*). Epilimnion depth is represented by a broken line.

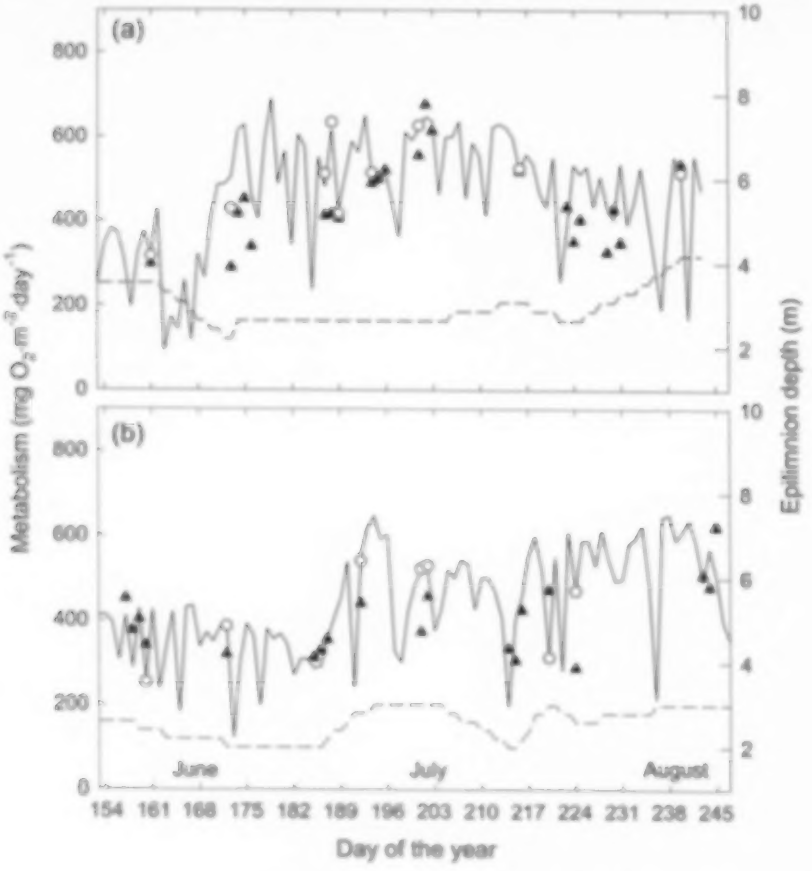
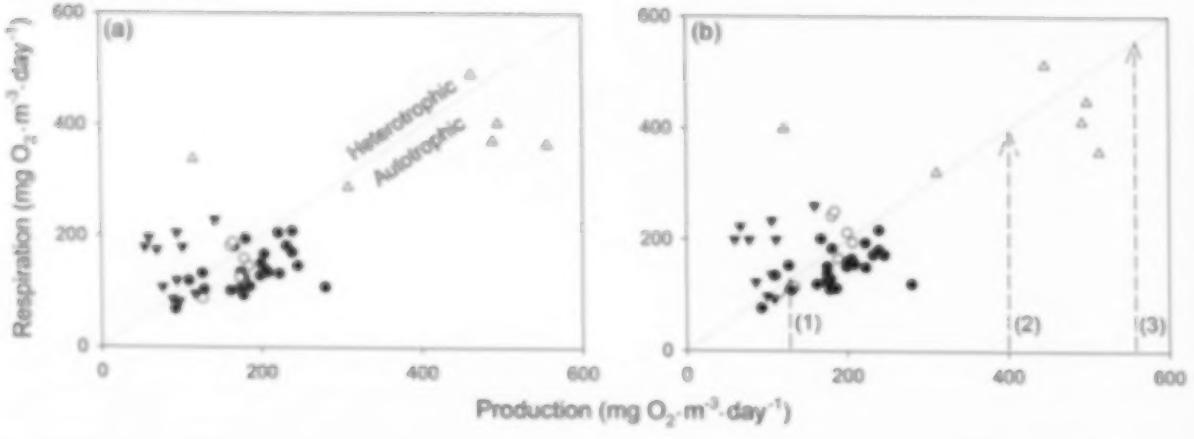


Fig. 4. Volumetric production and respiration before (*a*) and after (*b*) adjusting for the diel cycle of chlorophyll *a* and respiration for Lac Croche (solid circles), Ottawa River (open circles), St. Lawrence River (solid triangles), and Lac à l'Ours (open triangles). The arrows in panel (*b*) represent the threshold production for net heterotrophic systems reported by (1) Duarte et al. (2004) for an oligotrophic littoral ecosystem as 128 mg $O_2 \cdot m^{-3} \cdot day^{-1}$, (2) del Giorgio et al. (1997*a*) in marine and freshwater systems as 400 mg $O_2 \cdot m^{-3} \cdot day^{-1}$, and (3) del Giorgio and Peters (1994) in the mixed layer depth of a series of lakes as 558 and 733 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ when using their model 2 regression (not shown on the figure).



above 200 μmol photons·m^{-2}·s^{-1}. In our study, the aquatic systems presented attenuation coefficients (K) between 0.4 and 2.1 m^{-1}. Thus, irradiance levels above the threshold of 200 μmol photons·m^{-2}·s^{-1} at noon, using a clear sky model, would be contained within 1.2 and 6.2 m deep, corresponding to 20% to 130% of the mixing depth, respectively. A decrease in the surface irradiance due to either cloud coverage or different time of day would result in a decrease of the effect of photorespiration on the respiration estimates of the mixing depth. Thus, photorespiration would likely have accentuated the diel pattern of respiration, especially in ecosystems where phytoplankton represent a major component of the planktonic community, such as Lac Croche and Lac à l'Ours. However, the influence of the photorespiration on the total daily respiration is believed to be rather small.

Although diel variations in respiration rate were linked to temperature (see also Szyper et al. 1992), the rise in metabolic activity induced by a 1 to 2 °C rise in daily temperature cannot by itself account for the observed 50%–100% increase in daily respiration (Jugnia et al. 1998). For example, in a coastal system, planktonic respiration doubled as temperature rose from 15 to 28 °C, following an exponential function (Sampou and Kemp 1994; Smith and Kemp 1995). Similarly, the zooplankton community of an oligo-mesotrophic lake saw its growth rate tripled on a seasonal scale as the temperature increased from 2 to 20 °C (Carrick et al. 1992). It has thus been shown that temperature must at least double to explain a 50%–100% increase in daily respiration; other factors must therefore account for the diel variation in respiration.

Our estimations of bacterial abundance fell within the range reported in the literature for freshwater systems (Straskrabova and Fuksa 1982; Simon 1994; del Giorgio et al. 1996). Since bacterial respiration contributes between 10% and nearly 100% of planktonic respiration (Oleynik 1989; Findlay et al. 1992), bacterial metabolism should be related to the diel variation of planktonic respiration. Nevertheless, as previously reported in other freshwater systems (Straskrabova and Fuksa 1982; Simon 1994), we found no evidence of a diel cycle in bacterial abundance. Such a cycle has been observed in the ocean, however, exhibiting maximum values in daytime and minimum values at night (Fuhrman et al. 1985). The lack of relationship between respiration and bacterial abundance measured after SYTO-13 staining may be explained by the fact that SYTO-13 reveals all bacteria, regardless of their level of metabolic activity or viability (Choi et al. 1996; del Giorgio et al. 1997*b*; Berman et al. 2001). Others have observed diel cycles of bacterial growth rate, with an afternoon maximum (Straskrabova and Fuksa 1982; Chin-Leo et Benner 1991; Simon 1994), which corresponds to the respiration pattern we observed.

Chlorophyll *a* and respiration both underwent diel cycles, with a 50%–100% daytime increase in Lac à l'Ours and the Ottawa and St. Lawrence rivers. In oligotrophic Lac Croche, the chlorophyll *a* cycle was less pronounced, with a 9% (in 1998) and 17% (in 2000) daily rise from early morning values. Although the presence of a diel cycle of chlorophyll *a* has often been shown in oceanic systems (review of Sournia 1975; Le Bouteiller and Herbland 1982; Fuhrman et al. 1985), it was not detected in coastal systems (Harding et al. 1982*a*; Vandevelde et al. 1989). In freshwater systems, diel variations of chlorophyll *a* have been reported, but no periodicity nor diel pattern were observed (Glooschenko and Blanton 1977; Markager et al. 1992; Michard et al. 1995).

Although both respiration and chlorophyll *a* were cyclical over a 24 h period in almost all systems, with daytime maxima and nighttime minima, the cycles were not coincident and both variables were poorly related within systems. Across systems, chlorophyll *a* returned to minimal values during the first half of the night, whereas respiration remained high until the middle of the night. The relationship between respiration and chlorophyll *a* was significant in the Ottawa and St. Lawrence rivers and in Lac à l'Ours (R^2 = 0.39, $p < 0.001$). Although the relationship between the two variables is highly significant, the low explanation of the variance indicates a complex linkage probably involving the light-dependent algal production cycle, the destruction of phytoplankton and bacterioplankton by viruses, the periodic maxima in algal grazing by zooplankton (in lakes only) occurring early at night, and the labile organic carbon concentration reaching a maximum early at night. Furthermore, the production of labile carbon from the photodegradation of refractory allochthonous carbon may contribute to drive the respiration cycle through enhanced bacterial metabolism. Moreover, heterotrophic protozoans are known to contribute to a major proportion of bacterial production (about 40% in an oligo-mesotrophic lake, Carrick et al. 1992); they could thus play a role through their diel vertical migration to the cyclical respiration pattern.

Cumulative light intensity (PAR) over the 6 h preceding sampling was positively related to respiration for all systems (R^2 = 0.29 to 0.41; R^2 = 0.36 for all data combined, $p < 0.001$ for all relationships). The diel variation of primary production coincided with the daily cycles of light intensity and biomass and resulted in the accumulation, during the day, of freshly produced (autochthonous) organic carbon becoming available to zooplankton and heterotrophic bacteria. Note also that in Lac à l'Ours, the unusually low irradiance on 6 June 2000 corresponded to a lack of diel cycle in planktonic respiration. Diel respiration cycles therefore seem closely related to the diel variation in primary production.

Labile organic carbon production through interaction of UV with refractory allochthonous carbon (Kieber et al. 1989, 1990) is another possible source of carbon for heterotrophic processes. However, in Lac Croche, DOC concentrations remained stable throughout the summer (3.5 ± 0.1 mg·L^{-1}) and received little input from the watershed owing to low or absent runoff between July and September. Average daily volumetric respiration was around 150 mg O_2·m^{-3}·day^{-1} (4.7 mmol O_2·m^{-3}·day^{-1} or 3.75 mmol C·m^{-3}·day^{-1} based on a photosynthetic quotient of 1.25), which corresponds to about 375 mmol C·m^{-3} (daily respiration × 100 days) or 4.5 mg C·L^{-1} for the entire summer. Dissolved organic carbon of terrestrial origin is obviously insufficient to fulfill the organic carbon demand by heterotrophs in this system, and autochthonous carbon is therefore the most likely source of organic carbon supporting planktonic respiration.

Diel variation of phytoplanktonic productivity has been studied intensively, in the laboratory (Harding et al. 1981) and in marine ecosystems (MacCaull and Platt 1977; Harding et al. 1982*a*; Vandevelde et al. 1989), leading to a con-

senses that light-limited (α^B) and light-saturated (P_m^B) productivity present diel dependencies. Savidge (1988) reported a diel variation of the photosynthetic parameters but did not detect periodicity on a seasonal scale. Similar to our findings, MacCaull and Platt (1977) observed a diel variation of P_m^B with a midday maximum, whereas α^B showed no diel cycle from summertime sampling in a small marine inlet. Most studies detected a diel cycle for both photosynthetic parameters, with a daytime maximum and a nightly minimum (Harding et al. 1982*a*; Forbes et al. 1986; Vandevelde et al. 1989). In addition, diel periodicity appeared species-dependent; some diatom taxa presented considerable diel variations in P_m^B (up to 150% of nighttime values), whereas other species showed relatively constant values (Harding et al. 1981). Although phytoplankton species were not identified in this study, diatom populations are known to have limited blooming capacity in the well-stratified, low nutrient conditions found in temperate lakes during the summer season (Wetzel 1983). This might explain the small, but significant, increase at midday of P_m^B values, as reported from summertime sampling by MacCaull and Platt (1977).

Implications of diel chlorophyll and respiration cycles on metabolic balance estimates

Because diel variations of respiration and chlorophyll *a* are as large as seasonal variations, sampling protocols should be designed to eliminate the effect of diel variations when seasonal patterns are sought. Our results show that a single morning sampling underestimates both respiration and chlorophyll *a*, whereas a single sample taken at noon generally agrees better with the average of four daily measurements. In Chesapeake Bay, sampling between 0800 h and 1000 h provided a good agreement with the estimations of daily respiration derived from a diel cycle of measurements (Sampou and Kemp 1994). Our results stress the importance of standardizing sampling protocols to obtain comparable respiration rates.

Sampling time had little overall effects on daily estimates of primary production in Lac Croche, where no diel variation of α^B was detected. Maximum photosynthetic capacity P_m^B increased by 12.5% at midday compared with the early morning average. However, computed daily production using the midday P_m^B was only 9% higher than that obtained using morning P_m^B. The effect of diel variation of P_m^B on daily production estimates was even less pronounced when daily production was computed using a combination of morning, midday, and evening values of P_m^B, which was only 4% higher than that calculated using morning P_m^B. In marine systems, the difference between daily production estimated after accounting for diel variation of the photosynthetic parameters or estimated from a one-sample estimation ranged between 25% and 56% (MacCaull and Platt 1977), 4% and 74% (Harding et al. 1982*b*), and 15% and 70% (Vandevelde et al. 1989). Because marine systems usually exhibit lower attenuation coefficients than freshwater systems, light-saturated photosynthesis extends to correspondingly deeper sections of the water column. In such systems, a small variation in photosynthetic parameters would have a greater effect on the daily estimation of primary production than in more turbid fresh waters.

The diel cycle of respiration and chlorophyll only slightly affected the *P*:*R* ratio estimates. This is mainly due to the opposing effects of the respiration and chlorophyll diel cycles. Thus, as we observed an increase in respiration when sampling every six hours, chlorophyll concentrations, directly related to production, also increased with the higher temporal resolution sampling design. Notwithstanding this qualitative observation, a paired *t* test performed on all the *P*:*R* ratios estimated before and after accounting for the diel cycles of respiration and chlorophyll concentration showed a significant difference ($p < 0.001$)

P–*R* balance on a seasonal scale

Summertime estimates of the *P*–*R* balance accounting for the presence of diel chlorophyll and respiration cycles showed a positive *P*–*R* balance for Lac Croche during the summers of both 1998 and 2000, in agreement with earlier findings in a meso-oligotrophic lake (Berman et al. 2004), in a large oligotrophic lake under stratified conditions (Russ et al. 2004), and in the epilimnion of a clear-water lake (Coloso et al. 2008). These results do not support those of del Giorgio et al. (1997*a*), who proposed that oligotrophic systems (GPP less than 120 mg $C \cdot m^{-3} \cdot day^{-1}$ or 400 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ using a photosynthetic quotient of 1.25; Williams and Robertson 1991) are net heterotrophic ($P:R < 1$). Moreover, our results do not agree with the conclusions of Prairie et al. (2002), who suggested that systems with DOC concentrations exceeding 5 $mg \cdot L^{-1}$ should be net heterotrophic. In our study, the St. Lawrence River, which has the lowest DOC levels, was the only system consistently showing net heterotrophy.

The controversy as to whether oligotrophic systems are net autotrophic or net heterotrophic (del Giorgio and Peters 1994; Carignan et al. 2000; Duarte et al. 2004) could be in part explained by the finding that the metabolic status of oligotrophic lakes varies over the annual cycle (Biddanda and Cotner 2002; Russ et al. 2004). Over the years, many threshold values of primary production to reach balanced metabolism have been proposed. In our study, two oligotrophic and two mesotrophic ecosystems presented a mix of net autotrophic and net heterotrophic conditions. We found a high percentage of net autotrophic samples below the threshold production proposed by both del Giorgio and Peters (1994) and del Giorgio et al. (1997*a*), respectively, 62.5% and 61.4% of net autotrophic samples. The threshold production proposed by Duarte et al. (2004) for a littoral system using an oxygen method agreed well with our study, with only 25% of our samples belonging to net autotrophic conditions below their threshold value, mainly from St. Lawrence River (average *P*:*R* ratio of 0.68 for the St. Lawrence, average *P*:*R* ratio of 0.65 in Bay of Blanes; Duarte et al. 2004). In their paper, Duarte et al. (2004) explain the net heterotrophic conditions as resulting from allochthonous inputs from urban runoff supporting heterotrophic-dominated planktonic communities (biomass of heterotrophs being 59% of total biomass). In our study, the St. Lawrence River showed high bacterioplankton concentration, which might explain the slight net heterotrophic conditions found in this ecosystem.

In future work, time of sampling for metabolism measurements should be carefully chosen to ensure good estimates of daily metabolism, favouring late morning sampling or preferably dual samplings (morning and late afternoon). The

methodological implication of our study on diel variation of metabolism measurements might also alter conclusions from previous work, where for the most part sampling was performed in early morning and might result in underestimation of both respiration and chlorophyll *a* concentration. More importantly, our work presents the critical status oligotrophic systems play in the assessment of the global carbon cycle. It has been shown that inland waters play a major role in the global carbon cycle (Cole et al. 2007) and that oligotrophic ecosystems contribute to a large portion of the global aquatic systems (Lieth 1978). The new field data provided here promote a low threshold production for balanced metabolism and thus increase the autotrophic contribution of oligotrophic freshwater systems to the pool of aquatic ecosystems.

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Bioenergetics, overcompensation, and the source–sink status of marine reserves

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Abstract: One of the hypothesized functions of marine protected areas (MPAs) is to serve as sources of biomass, with biomass spilling over from the reserve into neighbouring, harvested areas. We argue that the net larval flow (from or to the marine reserve) depends on between-area differences in the population-level biomass production rate, whereas the direction of adult flow depends on differences in the biomass standing stock. Hence, an important question is whether population-level biomass production increases (overcompensation) or decreases (undercompensation) with increased per capita mortality. We show that in a consumer–resource context, the source–sink status of an MPA may depend on the details of the individual-level bioenergetics, as well as on the dispersal rates of larvae and adults. We compare two classic bioenergetic models (net-production vs. gross-production allocation). The net-production model predicts that population-level reproduction may increase with mortality (overcompensation), whereas gross-production allocation always results in undercompensation. We show that models often implicitly assume gross-production allocation, thus potentially overestimating the capacity of MPAs to source unprotected areas. We briefly discuss results of two other models (a simplified, logistic model and a size-structured model), suggesting that the relation between overcompensation and the larval sink status of MPAs is general.

Résumé : Une des fonctions présumées des zones de protection marine (« MPA ») est de servir de source de biomasse, cette biomasse débordant alors de la réserve vers les zones exploitées adjacentes. Nous soutenons que le flux net de larves (depuis ou vers la réserve marine) dépend des différences entre les deux zones du taux de production de la biomasse au niveau de la population, alors que la direction du flux des adultes dépend des différences de biomasse des stocks. Cela soulève une question importante à savoir si la production de biomasse au niveau de la population augmente (surcompensation) ou diminue (sous-compensation) en fonction d'une augmentation de la mortalité par individu. Nous démontrons que, dans un contexte de consommateurs et de ressources, le statut de source ou de piège d'une MPA peut dépendre des détails de la bioénergétique au niveau individuel, mais aussi des taux de dispersion des larves et des adultes. Nous comparons deux modèles bioénergétiques classiques (allocation de la production nette ou de la production brute). Le modèle de production nette prédit que la reproduction au niveau de la population peut augmenter en fonction de la mortalité (surcompensation), alors que l'allocation de la production brute entraîne toujours une sous-compensation. Nous montrons que les modèles présupposent souvent une allocation de la production brute, surestimant ainsi la capacité des MPA d'alimenter les zones non protégées. Nous discutons brièvement des résultats de deux autres modèles (un modèle logistique simple et un modèle structuré en fonction de la taille) et nous croyons que la relation entre la surcompensation et le statut des MPA comme pièges pour les larves est une relation générale.

[Traduit par la Rédaction]

Introduction

Marine reserves (or marine protected areas, MPAs hereafter) introduce a spatial heterogeneity into ecosystems that is caused by spatial variation in mortality rates of harvested populations. This results in an imbalance between protected and unprotected areas not only in terms of standing biomass of populations in the food web (Halpern and Warner 2002; Micheli et al. 2004*b*) but also, by consequence, in terms of ecological interactions, such as competition for resources, predation, etc. This imbalance is the driving force behind the potential of marine reserves to serve as biomass sources (through spillover) for the harvested areas, possibly leading to equal or even enhanced yield compared with conventional, effort-based fisheries management (Mangel 1998; Neubert 2003; Hart 2006). This simple vision of marine reserves suggests that two aspects are important for the dynamics of MPAs and surrounding areas: (*i*) the local ecological response of the food web to the presence or absence of harvesting, and (*ii*) the exchange of individuals through migration (Walters 2000; Mangel and Levin 2005). Mathematical models have been used to investigate the consequences of different assumptions on these two aspects for

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both the economic and conservational implications of MPAs (reviewed in Gerber et al. 2003; Baskett et al. 2007; Pelletier et al. 2008), including the roles of predation, competition, and mutualism (Baskett et al. 2007), spatial processes (Botsford et al. 2001; Guichard et al. 2004), juvenile–adult stage structure (St. Mary et al. 2000), and density-dependent growth (Gårdmark et al. 2006).

In this paper, we show that a fundamental but usually implicit assumption concerning individual-level energy allocation affects both aspects (*i*) and (*ii*) in a potentially important way, which has been ignored in most previous modelling work. We study two alternative, well-known models for individual-level bioenergetics. A bioenergetics model answers the question as to how much energy is allocated to growth, reproduction, and maintenance, given a quantity of food in the environment. In the literature, different models of energy allocation exist, which can be divided into two classes: gross-production allocation and net-production allocation. The distinction between the two classes is that reproduction is supposed to be proportional to the energy intake rate either before or after maintenance costs have been substracted. By consequence, the allocation models assume different behaviour at low food conditions: in the gross-production model, individuals reproduce as long as the food concentration is positive, whereas in the net-production model, there is a threshold food concentration below which individuals stop reproducing (Gurney et al. 1996). Simple MPA models formulated in terms of ordinary or partial differential equations (ODEs or PDEs, respectively) are often based on Lotka–Volterra type models (Neubert 2003; Steele and Beet 2003; Baskett et al. 2006), without explicitly considering bioenergetics. We show that such models are equivalent to an implicit assumption of gross-production allocation.

In the context of size-structured population models, Gurney et al. (1996) have shown that the individual-level energetics influence by which demographic process populations are regulated (i.e., reproduction, maturation, or survival limitation). The relevance of such individual-level assumptions in the context of MPAs is their implications for the local ecological response to the presence or absence of harvesting (aspect (*i*) above); the food web response will depend on how individuals react to (changes in) their environment. To illustrate this point, in this paper we study the consequences of different bioenergetic assumptions embedded in a simple food web consisting of a harvested consumer population (without size structure) and its resource population, based on the model of Yodzis and Innes (1992). Space is divided into a protected area and a harvested area, between which consumers (but not the resource) are assumed to migrate. Our model allows the dispersal rates of larvae and adults to be tuned independently. Density dependence results from competition for, and depletion of, the resource. The resource density inside and outside the MPA influences the energy budget and hence the respective rates of biomass growth and reproduction of the consumers in the two areas.

We thus address the question of how density-dependent food limitation, and its consequences for growth and reproduction, will affect projected effects of spillover from marine reserves. One of the hypothesized functions of marine protected areas (MPAs) is to serve as sources of biomass, with biomass spilling over from the MPA into neighbouring, harvested areas. We show that in a consumer–resource context, the source–sink status of the MPA may depend on the details of the individual-level bioenergetics, as well as on the dispersal rates of larvae and adults. We investigate the robustness of our model results by comparing the results with two alternative models: a simpler one (based on the logistic model) and a more complex one (a size-structured model). The latter is of relevance as the origin of the energy allocation models lies in the context of size-structured models. In particular, size-structured models allow for different kinds of population regulation (maturation vs. reproduction limitation; de Roos et al. 2007), which may have consequences for the success of marine reserves.

The model

We model the biomass dynamics of a harvested fish population and its forage base, taking into account the consumer–resource interaction between these two populations, as well as a simple representation of the bioenergetics underlying the fish biomass dynamics. To introduce the notation, we first present a spatially undivided version of the model, based on Yodzis and Innes (1992). The biomass dynamics of the fished stock (C for consumer) and its forage base (R for resource) are modelled as follows:

$$\frac{dC}{dt} = g(R)C + b(R)C - \delta C \tag{1}$$

$$\frac{dR}{dt} = \rho(R_{max} - R) - j(R)C \tag{2}$$

where C and R are biomass per unit of sea surface area. The fish dynamics are governed by biomass production though somatic growth $g(R)$ and birth $b(R)$, which depend on the current food density R, and mass-specific fish mortality, which is denoted by δ. The forage base R follows semi-chemostat dynamics with dilution rate ρ and maximum resource density R_{max}. The function $j(R)$ is the mass-specific functional response of fish.

Note that $g(R)$ and $b(R)$ are production rates per unit of standing stock biomass. Below we make frequent use of the population-level production rates, which are defined as $G = g(R)C$ and $B = b(R)C$, respectively.

Energy budget: biomass growth, reproduction, and maintenance

The birth and somatic growth terms ($b(R)$ and $g(R)$) are based on simple bioenergetic considerations, based on the model of Yodzis and Innes (1992). Their model assumes that the production rate of biomass depends on the mass-specific ingestion rate ($j(R)$), the loss rate due to maintenance costs (m), and the loss rate due to mortality (δ):

$$\frac{dC}{dt} = C[\sigma j(R) - m - \delta] \tag{3}$$

where $1 - \sigma$ is the fraction lost as feces and urine between ingestion and the metabolizable energy level. We assume that the consumer intake rate, $j(R)$, follows either a type-I or a type-II functional response, i.e., respectively,

$$(4) \qquad j(R) = aR \quad \text{or} \quad j(R) = \frac{R}{R_h + R} j_{max}$$

where a is an attack rate, R_h is the half-saturation density, and j_{max} is the maximum intake rate. The Yodzis and Innes model (eq. 3) may be a sufficient description of the bioenergetics for a single population (in spite of its simplicity in terms of the absence of size or stage structure (de Roos et al. 2007) or energy storage (Kooijman 2000)). Yet for spatially subdivided populations with larval dispersal, such as in the case of marine reserves, it is necessary to at least specify the allocation of resources between reproduction, maintenance, and growth, because we need to know which part of the biomass production will be redistributed in the form of larval dispersal over the spatial range of the population.

Net-production allocation vs. gross-production allocation

Maintenance, reproduction, and somatic growth all derive from the assimilation rate, $\sigma j(R)$ (Kooijman 2000). Two major types of energy allocation models have been proposed, which are referred to as net-production allocation and gross-production allocation (Gurney et al. 1996; Nisbet et al. 2004). The rate of net production per unit of biomass equals the difference between the energy intake rate and the loss rate due to maintenance, $\sigma j(R) - m$, whereas gross production equals $\sigma j(R)$. Under net-production allocation, it is assumed that a fraction κ of the net production rate is allocated to somatic growth, and the fraction $1 - \kappa$ is used for reproduction. For the net-production allocation model, the rates of biomass production through somatic growth (g) and reproduction (b) then equal

$$(5) \qquad \begin{aligned} g(R) &= (\sigma j(R) - m)\,\kappa \\ b(R) &= (\sigma j(R) - m)\,(1 - \kappa) \end{aligned}$$

For this model, it is necessary to specify what happens in case of starvation. In the MPA model specified below, it is possible that biomass flow increases local competition to such an extent that net production becomes negative in one of the areas. In this case, we assume that the birth rate b becomes zero, and the somatic growth rate g becomes equal to the (negative) net production rate. This amounts to assuming that in case of starvation, standing biomass is converted to cover maintenance.

Under gross-production allocation, it is assumed that a fraction κ of the gross production rate is used for both maintenance and somatic growth, and the fraction $1 - \kappa$ is allocated to reproduction, which is also referred to as the "kappa rule" model (Kooijman 2000). So for the gross-production allocation model, the rates of biomass production through somatic growth (g) and reproduction (b) are

$$(6) \qquad \begin{aligned} g(R) &= \sigma j(R)\kappa - m \\ b(R) &= \sigma j(R)\,(1 - \kappa) \end{aligned}$$

Note that a special rule for starvation is unnecessary in the gross-allocation model: in case of starvation, the birth rate is still positive, and standing biomass is used to cover maintenance. Although a special rule for starvation may be more realistic for any particular fish species, we choose to use this simple assumption.

An important difference between the net- and gross-production models is hence their behaviour at low food density: in the former model (eq. 5), reproduction and somatic growth stop simultaneously at a critical low food density, whereas in the latter model (eq. 5), growth stops but reproduction continues.

Note that for a spatially undivided population, the two models result in exactly the same total biomass dynamics, as all that matters is the sum of $b(R)$ and $g(R)$ (eq. 1). For both allocation models, we then obtain eq. 3.

We point out that in the gross-production allocation model, maintenance is equivalent to a local rate of biomass loss, similar to a mortality rate. At the population level, maintenance can hence be incorporated into a general loss term $\delta' = m + \delta$. MPA models that do not explicitly consider bioenergetics simply redistribute the population-level reproduction rate $B = b(R)C$ over protected and harvested areas, without discounting the maintenance costs. This amounts to the implicit assumption that maintenance costs are either absent or incorporated in the mortality loss term. In other words, ignoring bioenergetics corresponds to assuming implicitly gross-production allocation. Note also that assuming the absence of maintenance costs ($m = 0$) is equivalent to the gross-production model.

Spatial structure: marine reserve and fished area

Next we subdivide the consumer–resource system into a marine reserve and a harvested area. Adults (i.e., standing biomass) suffer a per-unit biomass harvesting mortality in the fished area, denoted by H. The protected area is assumed to cover a fraction x of the total area A. The fraction α of produced consumer larvae is assumed to migrate and to be equally redistributed between the areas. Adults migrate with rate β. Note that larval migration amounts to a redistribution of the birth rate, whereas adult migration amounts to a redistribution of standing stock biomass. The so-called larval pool equal redistribution (LPER) assumption is equivalent to setting $\alpha = 1$ and $\beta = 0$.

Variables and population-level rates pertaining to the protected and the harvested areas are denoted by indices 1 and 2, respectively. For example, the population-level birth rates are now defined as $B_1 = b(R_1)C_1$ and $B_2 = b(R_2)C_2$, respectively. Because all variables are expressed as densities per unit of sea surface area, the total biomass in the protected and fished areas equals C_1Ax and $C_2A(1 - x)$, respectively.

Biomass flow between areas: source and sink

The dynamics in the two areas depend on the biomass flow of larval and adult biomass. Assuming that from both areas, a fraction α of produced larvae enters a common dispersal pool, the rate of larval settlement in each area equals the sum of local larvae production, $(1 - \alpha)B_i$, and the inflow of larvae from the dispersal pool, $\alpha[xB_1 + (1 - x)B_2]$. This corresponds to the widely used LPER assumption. The larval settlement rate in the protected area is then, after simplicifation,

$$(7) \qquad S_1 = B_1 - \alpha(1 - x)(B_1 - B_2)$$

For the fished area, the equivalent rate is

$$(8) \qquad S_2 = B_2 + \alpha x(B_1 - B_2)$$

These equations show that the larval settlement rate exceeds the local birth rate in the area with the lowest local birth

rate. In other words, the net larval flow is always from the most productive to the least productive area. The net larval flow from the reserve to the harvested area (denoted by f_L) equals the difference between inflow and outflow in the harvested area, multiplied by the size of the harvested area:

$$(9)\quad f_L = \{\alpha[xB_1 + (1-x)B_2] - \alpha B_2\}(1-x)A = \alpha x(1-x)A(B_1 - B_2)$$

The flow is positive, i.e., from the reserve to the harvested area, if and only if the reserve is more productive (per unit of sea area) than the fished area ($B_1 > B_2$).

Similar to larval migration, adults are assumed to migrate with rate β and to enter a common dispersal pool, which is then equally redistributed. Although this way to model adult migration is less standard than for larvae, the resulting equation is completely analogous to the more commonly used diffusive model.

We obtain expressions similar to larval migration. For example, in the reserve, the rate of departing biomass equals βC_1 and the rate of incoming biomass equals $\beta[xC_1 + (1-x)C_2]$. The migration balance thus equals $-\beta(1-x)(C_1 - C_2)$ in the reserve and $\beta x(C_1 - C_2)$ in the harvested area. The net biomass flow due to adult migration is again found by multiplication of either of these by the corresponding surface area:

$$(10)\quad f_A = \beta x(1-x)A(C_1 - C_2)$$

The flow is positive, i.e., spillover from the reserve to the harvested area, if and only if the biomass density is highest in the reserve ($C_1 > C_2$).

Note that the direction of the larval flow depends on differences in the biomass production rate, whereas the direction of adult flow depends on differences in the biomass standing stock. The two flows do not necessarily point in the same direction. The net total biomass flow equals their sum $f_{tot} = f_L + f_A$.

The full MPA model

Extending eqs. 1–2 to two areas and substituting the migration terms, we obtain

$$(11)\quad \frac{dC_1}{dt} = G_1 + B_1 - \alpha(1-x)(B_1 - B_2) - \beta(1-x)(C_1 - C_2) - C_1\delta$$

$$(12)\quad \frac{dC_2}{dt} = G_2 + B_2 + \alpha x(B_1 - B_2) + \beta x(C_1 - C_2) - C_2(\delta + H)$$

$$(13)\quad \frac{dR_1}{dt} = \rho(R_{max} - R_1) - f(R_1)C_1$$

$$(14)\quad \frac{dR_2}{dt} = \rho(R_{max} - R_2) - f(R_2)C_2$$

The dynamics of the fish populations (C_1 and C_2) are the sum of four terms: local growth of standing biomass, larval production and redistribution, adult migration, and mortality. Note that we assume that the food base (R_1 and R_2) does not migrate.

Parameter values

The default parameter values of the consumer–resource model are based on the equivalent but stage-structured model of de Roos et al. (2007): $j_{max} = 6$, $m = 1$, $\sigma = 0.7$, $\rho = 0.5$, $R_h = 1$. The natural mortality rate is assumed to be $\delta = 0.1$, which represents a typical value on a per-year basis for marine fish populations (Andersen and Ursin 1977). We have, however, tested the model behaviour over a range of δ up to 1. We obtain qualitatively similar results in all cases, although increasing δ decreases standing stock biomass and adult flow and makes the larval flow less negative. In our simulations presented below, we vary the harvesting rate H. We note, however, that rough estimates of harvesting mortality fall in the range of 2–10 times the natural mortality rate (Pope et al. 2006). With a mortality rate of $\delta = 0.1$, appropriate harvesting rates (H) thus fall in the range of 0.2 to 1. The larval migration parameter α is varied between 0 and 1 (note that α is a proporion). The adult migration parameter β should be interpreted relative to the mortality parameter: on average, an unharvested individual is expected to migrate β/δ times during its life time. We vary β between 0 and 1.

Results

Consequences of choice of allocation model

To understand how the MPA system reacts to harvesting mortality, it is instructive to first analyse the how the equilibrium of the single-area system (eqs. 1–2) without dispersal depends on the level of mortality, δ. Recall that for a single population, the allocation into growth and reproduction is irrelevant, as it is their sum that appears in eq. 1.

To simplify the analysis, we initially assume a linear functional response, which does not qualitatively influence the results (see below). For the single-area system (eqs. 1–2), the equilibrium equals

$$(15)\quad \hat{C} = \frac{\rho(\sigma a R_{max} - m - \delta)}{a(m+\delta)}$$

$$(16)\quad \hat{R} = \frac{m+\delta}{\sigma a}$$

These equations show that $\hat{C}$ decreases and $\hat{R}$ increases with mortality, because $\partial\hat{C}/\partial\delta < 0$ and $\partial\hat{R}/\partial\delta > 0$. The fish population goes extinct when mortality exceeds the value

$$(17)\quad \delta_{ext} = \sigma a R_{max} - m$$

The equilibrium (eqs. 15–16) is the same for the two allocation rules (eqs. 5–6). However, this is not true for the steady-state total reproduction rate, denoted by $\hat{B}$. With net-production allocation,

$$(18)\quad \hat{B} = \delta(1-\kappa)\hat{C}$$

with $\hat{C}$ as defined in eq. 15, whereas with gross-production allocation,

$$(19)\quad \hat{B} = (m+\delta)(1-\kappa)\hat{C}$$

An important difference between the allocation rules is the resulting relation between the total reproduction rate and (mass-specific) mortality. By substituting eq. 15 into eq. 19,

we find that with gross-production allocation, the reproduction rate necessarily decreases with mortality, i.e., $\partial\hat{B}/\partial\delta < 0$. Yet with net-production allocation, the curve of $\hat{B}$ over mortality is dome-shaped; it first increases between $\delta = 0$ and a critical mortality rate δ_{crit}, after which it decreases to zero at δ_{crit}. The critical mortality rate is as follows:

$$(20) \qquad \delta_{crit} = -m + \sqrt{\sigma\alpha R_{max}\, m}$$

This equation shows that with $m > 0$, the population-level birth rate can increase with mortality, even though the equilibrium biomass $\hat{C}$ always decreases with mortality. We refer to such a postive relation between total reproduction and mortality as "overcompensation".

The explanation of the overcompensation lies in (*i*) the resource dependence of the reproduction rate and (*ii*) the balancing of the birth and mortality rates in equilibrium. Increasing mortality decreases competition for the resource, which increases the reproduction rate per unit of biomass. Yet the total (population) reproduction rate may still decrease if the standing biomass decreases faster than the fecundity increases. This is the case when reproduction is proportional to the intake rate and hence to the resource density, as in the gross-production model. However, when reproduction is proportional to the net-production rate, by contrast, fecundity can increase much faster than the resource density itself. This becomes obvious in the limit of zero mortality. The equilibrium condition $dC/dt = 0$ requires that net production balances the death rate, i.e., $\sigma j(\hat{R}) - m = \delta$, which means that for $\delta = 0$, the net-production rate is zero. This, in turn, means that the total reproduction rate $\hat{B}$ is zero for $\delta = 0$. $\hat{B}$ then necessarily increases with δ.

In summary, overcompensation is never found in the gross-production model, whereas it occurs in the net-production model whenever the background mortality is relatively low ($\delta < \delta_{crit}$). This result is true also for a type-II functional response, but the equation of δ_{crit} is very ugly in that case. A graph of δ_{crit} for a type-II functional response, plotted against m (Fig. 1), shows that overcompensation is likely to occur with plausible parameter values; across a range of taxa, the mortality rate appears to be of the order of 10% of the maintenance rate (de Roos et al. 2007). Figure 1 illustrates that for this estimation, most combinations of δ and m fall in the region of overcompensation. Very similar results are obtained when varying the parameters R_{max}, σ, and R_h (data not shown).

Implications for the MPA model

The analysis of the single-area model gives an idea of how the MPA model will behave. As a simple thought experiment, compare the two areas of the MPA model, without any migration and with a low harvesting rate. The analysis tells us that the standing biomass will be lower in the harvested area, for either allocation model. Assuming gross-production allocation, we also know that the total birth rate will be lower in the harvested area. So we expect that allowing either larval migration between the two areas or adult migration will result in a biomass flow from the reserve into the harvested area (i.e., $f_L > 0$ and $f_A > 0$).

Next, consider the net-allocation model. Because of the overcompensation, the total birth rate is then likely to be higher in the harvested area than in the MPA. Larval migration is hence likely to result in a biomass flow from the harvested area into the reserve ($f_L < 0$). With net-production allocation, we therefore expect the MPA to be a biomass sink for larvae.

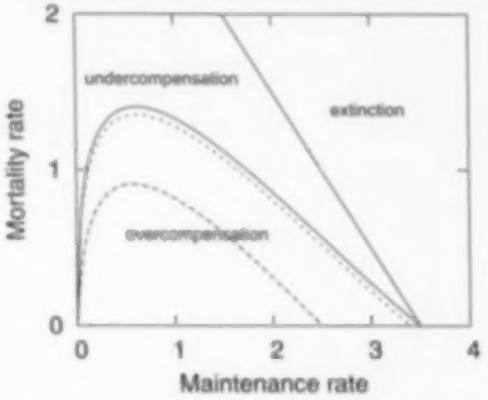


Fig. 1. The regions of overcompensation, undercompensation, and extinction in m,δ-parameter space for the spatially undivided model (eqs. 1–2) assuming net-production model and a type-II functional response. Without maintenance ($m = 0$), being the analogous case of gross production, we obtain undercompensation for all values of the mortality rate except zero. For comparison with the MPA model (eqs. 11–14), we have indicated for the MPA model with $\alpha = 1$ and $\beta = 0$, the curves where larval flow $f_L = 0$, assuming $F = 1$ (broken line) or $F = 0.1$ (dotted line), respectively. Parameters: $\kappa = 0.5$, $j_{max} = 6$, $R_h = 1$, $\sigma = 0.7$, $\rho = 0.5$, $R_{max} = 5$, $x = 0.3$.

However, these expectations should be checked with analysis of the full MPA model, as the steady state of the full model with migration does not necessarily conform to our intuition based on the limiting case of no migration.

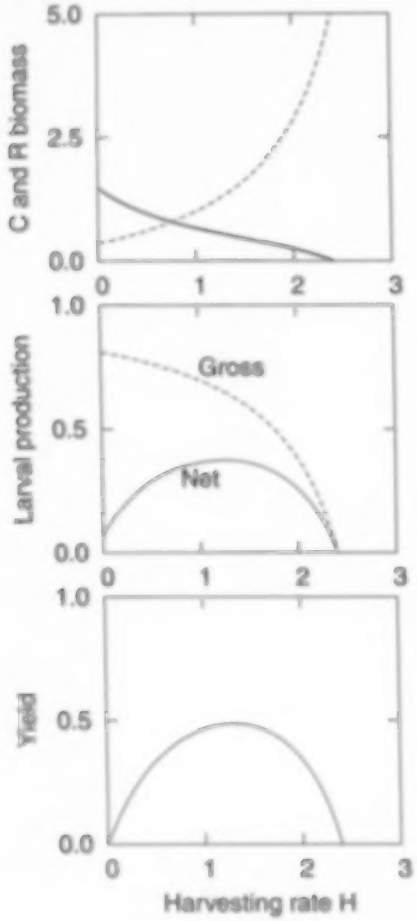
The MPA model: source–sink status of the reserve

Here we study the dynamics of the full MPA system (eqs. 11–14) assuming a type-II functional response. We compute the steady state of the MPA model over a range of the harvesting rate H (similar to Fig. 2), with the MPA being 30% of the total area ($x = 0.3$).

Model analysis confirms the intuition on the gross-production allocation model (Fig. 3). Assuming larval migration only ($\alpha = 1$, $\beta = 0$), the standing biomass decreases with H in both the protected and harvested areas, but is consistently higher in the protected area ($C_1 > C_2$). The same is true for the population birth rates (i.e., undercompensation and $B_1 > B_2$), despite increasing resource density in both areas. The resulting biomass flow is positive; the MPA is a source of larval production. Note also that the MPA prevents the harvested population from going extinct at high harvesting rates (cf. Fig. 2).

Model analysis also confirms the intuition on the net-allocation model (Fig. 4). The populaton birth rate in the harvested area displays the typical pattern of overcompensation (cf. Fig. 2). By contrast, the birth rate in the protected area decreases and is zero over a considerable range of H. In this range, the consumers in the MPA are

Fig. 2. Steady state of the spatially undivided version of the model (eqs. 1–2). Top panel: consumer (C; solid line) and resource (R; broken line) biomass density. Middle panel: population birth rate b for the net-production (solid line) and gross-production (broken line) models. Bottom panel: yield. All quantities are per unit of surface area. Parameters: $\kappa = 0.5$, $f_{max} = 6$, $R_0 = 1$, $m = 1$, $\sigma = 0.7$, $\delta = 0.1$, $\rho = 0.5$, $R_{max} = 5$.



starving due to severe resource competition. Throughout the range where $B_1 < B_2$, the larval flow is negative (Fig. 4). The reserve is thus a larval sink for harvesting rates up to the point at which the harvested population would go extinct in the absence of an MPA (cf. Fig. 2). Beyond this point, the fished stock is practically extinct, and the MPA becomes a source of larvae, saving the harvested population from true extinction.

In the marine reserve, the biomass initially increases with H, mirroring the pattern of larval production in the harvested area (Fig. 4). Although the resource in the exploited area increases gradually with H, converging to its carrying capacity at very high H, in the reserve, the resource first decreases with H, showing that competition for food intensifies in the MPA. In fact, the strong inflow of larvae causes an unsustainably high biomass density in the MPA: severe resource competition leads to starvation, most notably resulting in the complete absence of reproduction inside the MPA.

The idea is thus that overcompensation in the harvested area causes the MPA to be a larval sink. We test this idea by drawing the curve for which the larval flow is zero (Fig. 1), which delimits the region in which the MPA is a sink (i.e., below the broken or dotted curves). The figure shows that with decreasing H, the sink region approximates the region of overcompensation, thus confirming the idea. Note that with increasing H, the sink region becomes smaller, which reflects the fact that the sink status of the MPA depends on H and is obtained for small and intermediate H only (Fig. 4).

The effect of adult migration

Allowing adults to migrate ($\beta > 0$) results in an additional biomass flow, which is always directed from the MPA to the harvested area, because $C_1 > C_2$ (cf. eq. 10). Here we study the effect of adult migration on the larval flow (f_L) and total flow (f_{tot}).

Consider the example with $\alpha = 1$ and $\beta = 0.5$ (Fig. 5). (Note that $\beta/\delta = 5$, meaning that unharvested adults migrate on average five times in a life time.) The flow of standing biomass changes the pattern of consumer biomass in the reserve; C_1 now decreases monotonically with H. The adult flow releases the resource competition in the MPA, allowing for reproduction in that area. Initially, however, reproduction is still higher in the harvested area ($B_1 < B_2$), resulting in negative larval flow up to $H \approx 3$. In this range, the MPA is hence a sink for larvae, but a source of adults. Overall, the MPA is a source of biomass ($f_{tot} > 0$).

Next we study the effect of adult migration on the source–sink status of the MPA systematically (Fig. 6*a*). Figure 6 depicts the regions in H,β-parameter space where the MPA is a sink of total biomass and (or) larvae. These zones are delimited by the curves of $f_L = 0$ and $f_{tot} = 0$. The curve of $f_L = 0$ is almost vertical, indicating that the rate of adult migration has little influence on the sign of the larval flow. The MPA is a larval sink even for very high values of β. By contrast, the total biomass flow depends strongly on β: increasing adult migration reduces the range of H with negative total flow. The region marked "S" shows that starvation in the MPA, and hence zero reproduction, is restricted to low adult migration.

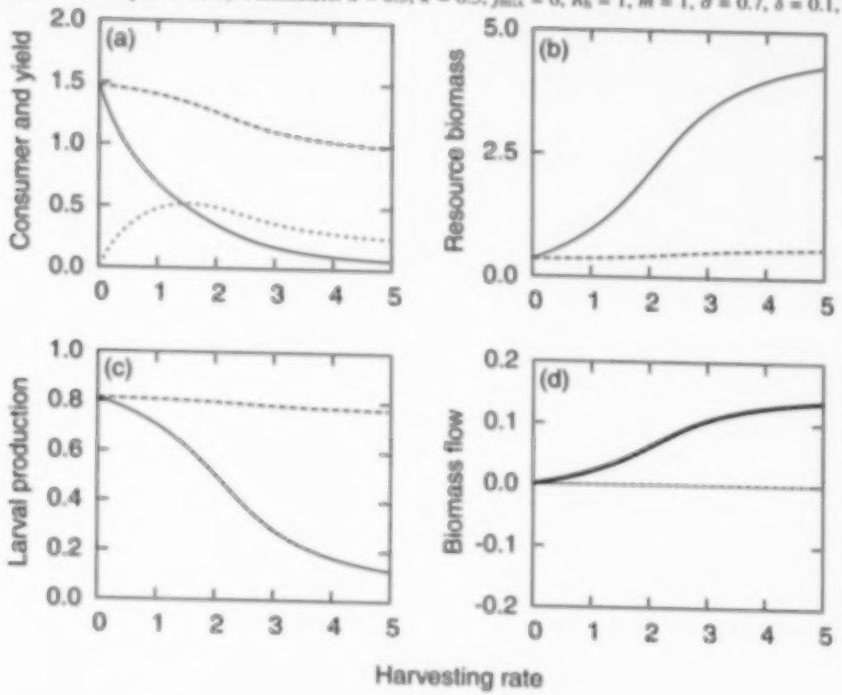
The effect of larval migration

The effect of changing the proportion of dispersing larvae (α) mirrors the effect of changing adult migration. For fixed adult migration ($\beta = 0.1$), reducing α has no influence on the sign of the larval flow (Fig. 6*b*). As expected, the total biomass flow becomes positive when larval migration becomes very small. Starvation is limited to very high larval migration.

The effect of the size of the MPA

The relative size of the reserve (x) determines the relative contribution of the two areas to the biomass flows; increasing x hence decreases the potential effect of overcompensation, which occurs in the harvested area only, on the larval flow. Changing x may influence the direction of the larval flow (f_L); if the MPA is larger, the larval flow is negative

Fig. 3. The equilibrium of the MPA model (eqs. 11–14) with gross-production allocation and larval migration only ($\alpha = 1$, $\beta = 0$). In *a*, *b*, and *c*, broken lines represent the marine reserve; solid lines, the harvested area. (*a*) Consumer population biomass and yield (dotted line); (*b*) resource biomass; (*c*) larval production $\bar{B}_1$ and $\bar{B}_2$; (*d*) net biomass flow (thick solid line; equal to larval flow) from MPA to harvested zone and adult flow (dotted line; equal to zero). Parameters: $x = 0.3$, $\kappa = 0.5$, $j_{max} = 6$, $R_b = 1$, $m = 1$, $\sigma = 0.7$, $\delta = 0.1$, $\rho = 0.5$, $R_{max} = 5$.



over a wider range of H (Fig. 7). At high H, small reserve size accentuates the "source" potential of marine reserves, as most of the locally produced larvae are lost to migration, thus reducing resource competition in the reserve and increasing the birth rate $\bar{B}_1$. However, at low and intermediate H, when overcompensation is strong in the harvested area (Fig. 4), small reserve size accentuates the "sink" potential of an MPA. With small x, the larval flow is largely determined by the harvested area such that overcompensation has a large impact on the dynamics in the MPA. By consequence, the starvation range is limited to small MPA size (Fig. 7).

Changing x does not, however, influence the sign of the total biomass flow (Fig. 7). This "inertia" is independent of the values of the other parameters (data not shown). The inertia can be understood as follows: when the total biomass flow is zero, each unit of sea area has a zero net flow. Given that the biomass inflow (larvae + adults) is the same for each unit of sea surface area, the biomass outflow per unit area (larvae + adults) must be the same in the MPA and the harvested area. Changing the relative contribution (x) to the common dispersal pool then has no effect whatsoever.

Alternative models

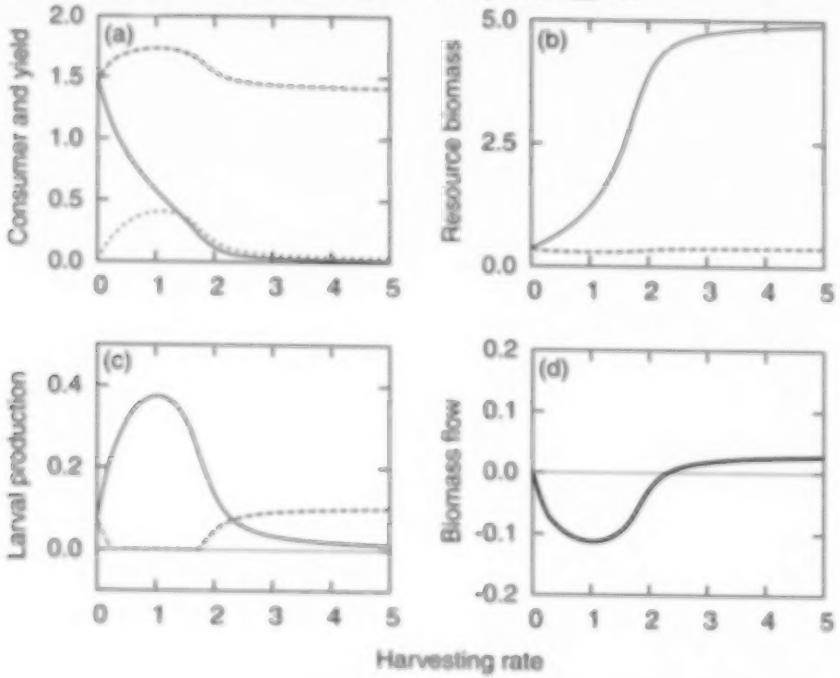
Size-structured population model

The allocation models studied in this paper were originally designed for size-structured population models (Gurney et al. 1996). In size-structured population models, density dependence may affect not only fecundity, settlement, and survival, but also maturation and individual growth. To illustrate the consequences of size structure, we analyse a well-studied size-structured population model in the context of our findings above.

The gross-production allocation model (eq. 6) first appeared as the kappa rule in a size-structured population model (Kooijman and Metz 1984). The Kooijman–Metz model has since been studied in various forms, and here we use the model published by de Roos and Persson (2002), but without predators. We extend their model by including an MPA and larval dispersal (analogous to eqs. (11–14) with $\alpha = 1$ and $\beta = 0$). In this model, individuals are born with size ℓ_b and mature at size ℓ_j. The gross-production model results in von Bertalanffy growth with asymptotic size $\ell_\infty R/(R_h + R)$. A model description (without MPA) can be found in de Roos and Persson (2002).

We do not present a full analysis of the size-structured model, but merely illustrate the implications of a juvenile life stage. In the size-structured model (without MPA), the consumer and resource biomass (Fig. 8*a*) follow the same pattern as in the unstructured model (cf. Fig. 2). Surprisingly, however, we find overcompensation (Fig. 8*b*), despite the gross-production allocation model. Based on the overcompensation, we thus expect a negative biomass flow (from the harvested area to the MPA) in the case of larval migration. Including an MPA in the size-structured model (with $x = 0.3$) confirms this expectation (Fig. 8*c*).

Fig. 4. The equilibrium of the MPA model (eqs. 11–14) with net-production allocation and larval migration only ($\alpha = 1$, $\beta = 0$). In *a*, *b*, and *c*, broken lines represent the marine reserve; solid lines, the harvested area. (*a*) Consumer population biomass and yield (dotted line); (*b*) resource biomass; (*c*) larval production $\bar{B}_1$ and $\bar{B}_2$; (*d*) larval flow f_l from MPA to harvested area (negative flow means reserve is larval sink). Parameters: $x = 0.3$, $\kappa = 0.5$, $j_{max} = 6$, $R_0 = 1$, $m = 1$, $\sigma = 0.7$, $\delta = 0.1$, $\rho = 0.5$, $R_{max} = 5$.



Which difference between the unstructured and structured models explains the found overcompensation? An important difference between unstructured and size-structured models is that the latter allows for different kinds of population regulation, depending on which demographic process (juvenile growth–maturation or adult reproduction) is most affected by intraspecific competition and hence limiting population growth. If a population is maturation-limited (as in Figs. 8*a*–8*b*), then increasing the mortality rate releases the competition among juveniles, and the adult biomass increases due to increased maturation (de Roos et al. 2007). In this case, we thus observe an overcompensation of the adult standing biomass: adult biomass increases with mortality (Fig. 8*a*, thin solid line), resulting in an overcompensation of the population-level reproduction rate (Fig. 8*b*). To demonstrate the crucial role of the juvenile life stage, we have rerun the model with $\ell_j < \ell_b$, such that individuals are born mature (Figs. 8*d*–8*f*). In this case, the population is necessarily reproduction-limited. Without a juvenile stage, the size-structured model predicts undercompensation (Fig. 8*e*). The corresponding MPA model displays a positive biomass flow (Fig. 8*e*), as expected from the undercompensation.

This simple example illustrates that maturation limitation in structured populations may lead to overcompensation in both adult biomass and the population reproduction rate, as shown by de Roos et al. (2007). The examples demonstrate that (*i*) overcompensation is not restricted to net-production allocation models and (*ii*) overcompensation in a model without MPA implies a negative larval biomass flow in the corresponding MPA model, whatever mechanism is causing the overcompensation. The size-structured model thus suggests that the association of overcompensation and negative biomass flow is general.

Logistic MPA model

Now that we have identified the role of overcompensation, we can check that it works in the simplest model that could produce such a result, the logistic growth model:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \tag{21}$$

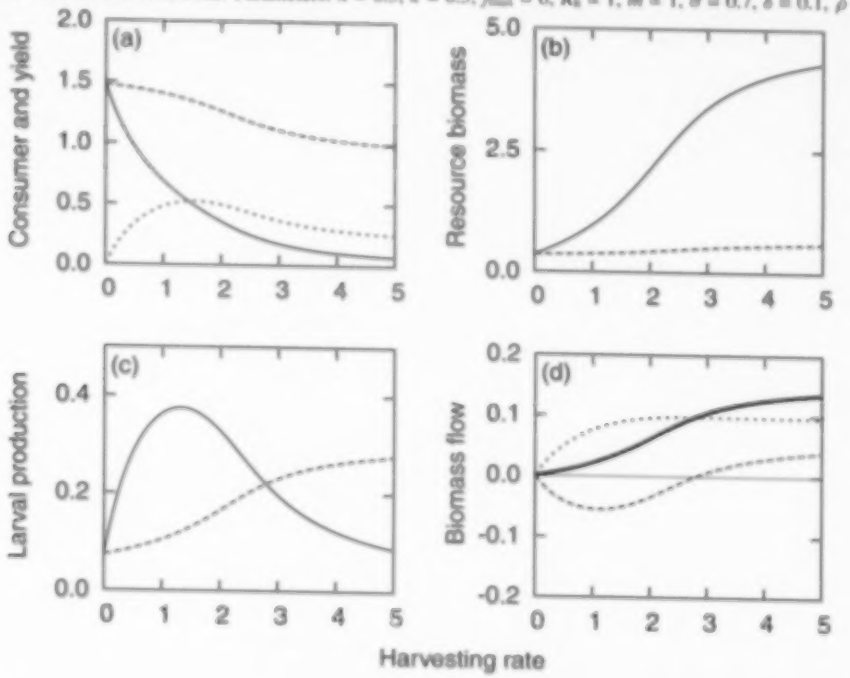
To be able to use the death rate as an independent parameter and to use the total birth rate as a model output, we have to decompose eq. 21 by making assumptions about how it reflects underlying per capita birth and death rates. As in the previous two models, we assume that density dependence affects the birth rate, whereas the death rate remains constant. By choosing $b - \beta N$ for the per capita birth rate and d for the death rate, we obtain,

$$\frac{dN}{dt} = N(b - \beta N - d) \tag{22}$$

which is equivalent to eq. 21 with $r = b - d$ and $K = (b - d)/\beta$.

The equilibrium population of eq. 22 equals $\hat{N} = (b - d)/\beta$. The population-level birth rate, defined as $\hat{B} = \hat{N}(b - \beta\hat{N})$, thus equals

Fig. 5. The equilibrium of the MPA model (eqs. 11–14) with net-production allocation and both larval and adult migration ($\alpha = 1$, $\beta = 0.5$). In *a*, *b*, and *c*, broken lines represent the marine reserve; solid lines, the harvested area. (*a*) Consumer population biomass and yield (dotted line); (*b*) resource biomass; (*c*) larval production $\hat{B}_1$ and $\hat{B}_2$; (*d*) larval flow (broken line), adult flow (dotted line), and net biomass flow (thick solid line) from MPA to harvested zone. Parameters: $x = 0.3$, $\kappa = 0.5$, $f_{max} = 6$, $R_h = 1$, $m = 1$, $\sigma = 0.7$, $\delta = 0.1$, $\rho = 0.5$, $R_{max} = 5$.



$$\hat{B} = d\,\frac{b-d}{\beta} \tag{23}$$

Because $\hat{B}$ goes to zero when d goes to zero, the logistic growth model clearly displays overcompensation for any value of b and β. Based on this observation, we expect that an MPA model that connects two logistic equations with larval dispersal will predict that the MPA is a larval sink. To check this prediction, we have extended eq. 22 by including a second harvested population and assuming LPER. We have computed its steady state and the corresponding direction of the larval biomass flow. The results are analogous to the ones obtained with the consumer–resource model above and confirm that the logistic model indeed predicts the MPA to be a larval sink for d below a critical background mortality rate (data not shown).

If, however, we assume that density dependence does not influence the birth rate but the death rate (by choosing $d + \beta N$ for the death rate and b for the birth rate), we obtain $\hat{B} = b(b - d)/\beta$, which corresponds to undercompensation. Thus, overcompensation requires that density dependence affects the birth rate.

Discussion

Our main result is that the source–sink status of marine reserves depends on the effect of the per capita mortality rate, including harvesting mortality, on the population-level reproduction rate (B). If this relation displays overcompensation (i.e., B initially increases with mortality), then any degree of larval migration results in a net biomass flow of larvae from the harvested area into the MPA, and the MPA is thus a sink of larval biomass. In the case of undercompensation, by contrast, the MPA is a source of larval biomass.

Why do MPAs become larval sinks? The explanation resides in intraspecific competition. Competition is more severe inside the MPA due to the reduced mortality rate (e.g., see the lower resource density R_1 in Fig. 3). When increased competition reduces the population-level reproductive output (i.e., overcompensation of B), then total reproduction is higher outside the MPA than inside. By consequence, the net larval flow will be towards the MPA. This, however, is not true for populations that display undercompensation of B (such as the gross-production model (eq. 6)).

For migration of standing biomass (as opposed to larval migration), our results suggest that marine reserves are usually a source of biomass, if it is assumed (as done here) that net migration goes from high-biomass to low-biomass areas. Larval and adult flow may thus be in opposite directions, and the net effect obviously depends on the relative rates of larval and adult migration. It should be noted, however, that in size-structured populations, adult biomass may itself display overcompensation, i.e., increase with mortality (de Roos et al. 2007). This may hence cause an MPA to be a sink of adult fish.

The main work presented here concerns the effect of alternative bioenergetic models on the source–sink status of MPAs. The conclusion is that gross-production allocation results in undercompensation of the population reproduction

Fig. 6. The effect of migration on the source–sink status of the MPA model (eqs. 11–14) with net-production allocation. Top panel: the effect of adult migration (β) for fixed larval migration ($\alpha = 1$). Bottom panel: the effect of larval migration (α) with fixed adult migration ($\beta = 0.1$). Solid lines indicate zero larval flow ($f_L = 0$) and zero total biomass flow ($f_{tot} = 0$). The dotted line ($\hat{B}_1 = 0$) delimits the region with starvation in the MPA (zone S). Zones: I, MPA is sink of larvae and of net biomass; II, MPA is sink of larvae but source of net biomass; III, MPA is source of larvae and of net biomass. Parameters: $x = 0.3$, $\kappa = 0.5$, $j_{max} = 6$, $R_0 = 1$, $m = 1$, $\sigma = 0.7$, $\delta = 0.1$, $\rho = 0.5$, $R_{max} = 5$.

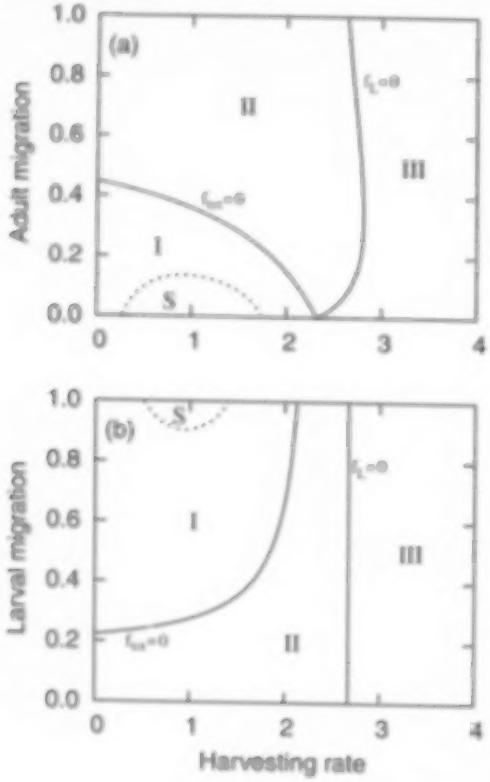
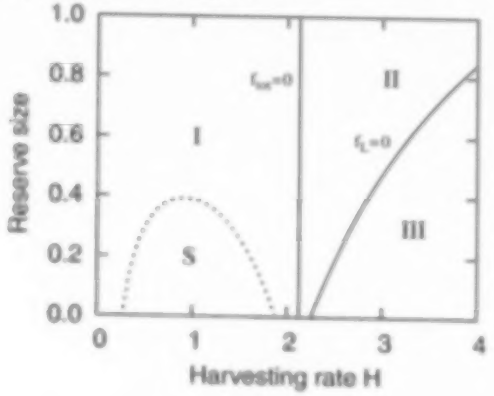


Fig. 7. The effect of relative reserve size (x) on the source–sink status of the MPA model (eqs. 11–14) with net-production allocation for $\beta = 0.1$ and $\alpha = 1$. Solid lines indicate zero larval flow ($f_L = 0$) and zero total biomass flow ($f_{tot} = 0$). The dotted line ($\hat{B}_1 = 0$) delimits the region with starvation in the MPA (zone S). Zones: I, MPA is sink of larvae and of net biomass; II, MPA is sink of larvae but source of net biomass; III, MPA is source of larvae and of net biomass. Parameters: $\kappa = 0.5$, $j_{max} = 6$, $R_0 = 1$, $m = 1$, $\sigma = 0.7$, $\delta = 0.1$, $\rho = 0.5$, $R_{max} = 5$.



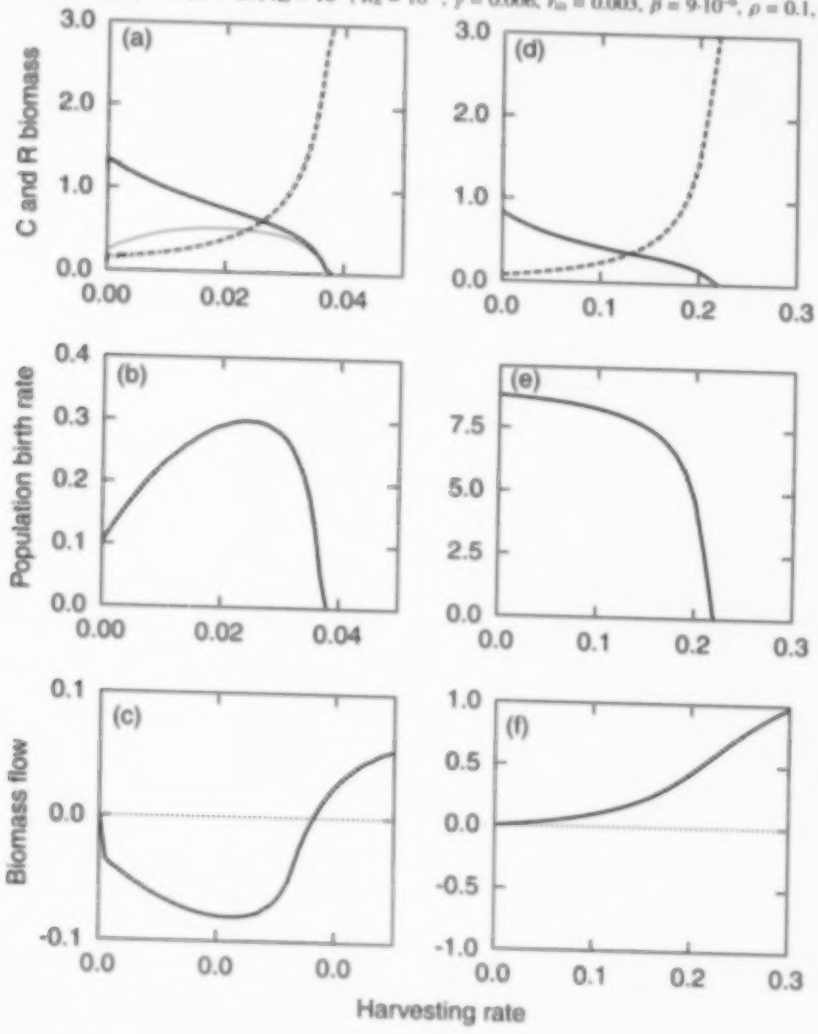
rate (and hence the MPA being a larval source), whereas net-production allocation is likely to result in overcompensation (and hence the MPA being a larval sink). However, our complementary analyses of a size-structured model, in which overcompensation is caused by a juvenile growth and maturation despite gross-production allocation, and a logistic model show that the connection between overcompensation and the larval-sink status of MPAs is more general and not limited to net-production models. In other words, overcompensation of the total reproduction rate causes the MPA to be a larval sink, whatever underlying mechanism causes the overcompensation.

Our main result depends on the combination of two factors: (*i*) overcompensation and (*ii*) larval dispersal. When both factors are present, the MPA is predicted to be a larval sink. Our study of three models shows that different mechanisms can underly the first of these factors. Common between all of them is a strong, density-dependent response of the population reproduction rate with increasing mortality. This response is stronger (at high population density) for net-production allocation than for gross-production allocation, explaining the different results for these two types of models. The logisic MPA model shows that overcompensation is found only when the logistic density dependence affects the birth rate (instead of the death rate). In the size-structured model, the birth rate depends not only on the fecundity (determined by gross-production allocation), but also on the age at maturation and survival to maturation, which are both density-dependent themselves. By consequence, the presence of a juvenile life stage accentuates the density dependence in the birth rate to such an extend that it results in overcompensation, despite gross-production allocation.

With respect to the second factor (larval dispersal), our results stress the need to distinguish between migration of newborns and of older individuals. Whereas the former amounts to a redistribution of biomass production, the latter amounts to a redistribution of biomass standing stock. Our main result depends critically on this distinction; only the redistribution of biomass production can result in the MPA being a sink, because the production can be higher in the harvested area than in the MPA. By contrast, standing biomass is generally higher in the MPA than in the harvested area (Halpern and Warner 2002; Micheli et al. 2004*b*) and therefore is likely to induce spillover into harvested areas. Although the distinction between larval and adult migration is common in MPA models, its implications for the source–sink status of marine reserves has not been identified before.

Here we note that the strictly negative relation between standing (adult) biomass and mortality found for unstructured models (eq. 15) does not necessarily apply to size-structured populations. Size-structured populations can be limited by different demographic processes, depending on which one of them is most affected by intraspecific compe-

Fig. 8. Steady state of a size-structured model with gross-production allocation. Left panels: fixed size at maturation ($\ell_j = 40$, $\ell_m = 80$). Right panels: absence of juvenile period ($\ell_j < \ell_b$, $\ell_m = 40$). Background mortality (left, $\mu = 0.01$; right, $\mu = 0.1$) and harvesting are size-independent. Top panels: consumer (solid lines) and resource (broken lines) biomass without MPA; adult biomass is represented by the thin solid curve. Middle panels: population birth rate ($\bar{B}$) without MPA. The left panel displays overcompensation despite gross-production allocation. Bottom panels: predicted biomass flow for corresponding MPA model with $x = 0.3$ and larval dispersal ($\alpha = 1$, $\beta = 0$). Biomass densities ($g \cdot m^{-3}$) and rates ($g \cdot m^{-3} \cdot day^{-1}$) are time-averaged over low-amplitude generation cycles (left panels). Parameters and notation are as in de Roos and Persson (2002): $\ell_b = 10$, $\alpha = 0.7$, $h_m = 10^{-4}$, $R_0 = 10^{-5}$, $\gamma = 0.006$, $r_m = 0.003$, $\beta = 9 \cdot 10^{-6}$, $\rho = 0.1$, $K = 3$.



tition (Gurney et al. 1996; de Roos et al. 2007). When a population is regulated by juvenile growth and maturation, then increasing mortality tends to increase the adult standing biomass. Although such overcompensation of adult biomass may in turn cause overcompensation of the population reproduction rate, in itself, it may cause a net migration of adults into the MPA. Hence, for maturation-limited populations, we predict that MPAs are likely to be general sinks of biomass, irrespective of the rates of larval and adult migration.

The review of Gerber et al. (2003) shows that previous models have often assumed either migration of larvae only (for example, models assuming LPER) or of adults only. Our work shows that it is in the case of LPER that overcompensation has the largest potential to cause MPAs to be biomass sinks. This result has not previously been found for the simple reason that the models used do not allow for factors (*i*) and (*ii*) to occur simultaneously. Many models in the literature do not explicitly take into account maintenance costs when computing the birth rate and hence implicitly correspond to the gross-production model (Neubert 2003; Baskett et al. 2006).

For example, the Lotka–Volterra competition models of Baskett et al. (2007) have the potential to display overcompensation, due to their equivalence to the logistic growth

model (see subsection Logistic MPA model). However, they assume that diffusion migration, i.e., migration of standing stock, results in a flow from high to low density and hence that the MPA is a source. The same is true for the models of Neubert (2003) and Steele and Beet (2003), which are equally based on the logistic growth model. The trophic models of Baskett (2006, 2007) and Baskett et al. (2006, 2007) also assume diffusion migration and hence do not need to distinguish between net- or gross-production allocation. However, we argue that in all of these models, larval flow is likely to be from the MPA to the harvested areas if larval dispersal and, in the trophic model, net-production allocation were assumed.

A second important result of our model is that the relative size of an MPA does not affect the sign of the total biomass flow between the MPA and surrounding areas. How can we understand this? This is a consequence of our assumption of a well-mixed, common dispersal pool. This assumption implies that for each unit of sea surface, the net biomass flow reflects the difference in local production of migrants and the global average production of migrants. Changing the MPA size (x) changes the relative contribution of areas with high and low migrant production and thus influences the average production of migrants. For example, if reserve size x is small and the reserve has a lower migrant production than the fished area, then increasing x will reduce the global average migrant production and hence reduce the influx of migrants into the MPA. Changing x thus has a quantitative effect on steady-state biomass flux (and standing biomass). This is not true, however, for the special case of zero net biomass flow. In this case, each unit of sea surface produces exactly the same amount of migrants. The local production and the global average are hence the same, irrespective of the size of the MPA. Changing the reserve size x has absolutely no effect on biomass flow or on the standing stock biomass.

Increasing reserve size is hence not likely to qualitatively improve the functioning of MPAs (in terms of the source–sink status of the MPA). Figure 7 illustrates that in the range of realistic harvesting rates (i.e., $H \approx 0.2$ to 1), the MPA is a biomass sink for both larvae and adults, no matter the size of the reserve. Only for heavily overexploited fisheries ($H > 2$) does the MPA function as a biomass source.

All of the models that we have discussed are continuous-time models. But what about discrete-time models? Many fisheries models are formulated in discrete time, based on the Ricker or Beverton–Holt models, so the question seems relevant. There is no reason to presume that for discrete-time models, the relation between overcompensation and the source–sink status of MPAs should not hold, in combination with the redistribution of larvae. For example, in the discrete time, predator–prey model of Micheli et al. (2004*a*), it is assumed that larvae enter a common dispersal pool. We have simulated their model, simplified by excluding the MPA, and found that the steady-state value of the variable L (equivalent to our $\bar{B}$), increases with fishing mortality for high values of the parameter a. Based on this observation, our results thus predict that in this model, the MPA can be a larval sink. Micheli et al. (2004*a*) did not, however, compute the direction of the larval flow.

More generally, for discrete-time models, whenever the recruitment rate displays overcompensation, larval redistribution is expected to result in larval flow from the harvested area to the MPA. This should be the case of models based on the Ricker model, which is the classical example of overcompensation, in contrast to the Beverton–Holt model, which displays undercompensation. However, the actual dynamics will depend on how the density dependence in such models is assumed to depend on the underlying vital rates (birth, death, growth, maturation).

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Density-dependent individual growth and size dynamics of central Appalachian brook trout (*Salvelinus fontinalis*)

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Abstract: Density-dependent aggression, emigration, mortality, and individual growth have been identified in wild lotic salmonid populations. However, most work is limited to fish in the genus *Salmo*, and few studies consider density dependency coupled with observations of energy acquisition. We attempt to quantify density-dependent growth, size, and energy acquisition for a population of brook trout (*Salvelinus fontinalis*) in Appalachia, where the species is food-limited and population densities are low. Brook trout populations were intensely monitored over the course of 2 years at several sites within a watershed in eastern West Virginia. Tagging provided specific growth estimates, and diet analyses allowed for estimation of energy acquisition relative to maintenance requirements for adult fish. Young-of-the-year (YOY) brook trout exhibited density-dependent size relationships, but the intensity of density dependency varied substantially among months, with progressively decreasing slopes. Adult individual growth and excess energy acquisition was density-dependent, but only during periods when temperatures were warm and energy intake was low. The strong influence of temporal variability in density dependence of YOY size, as well as adult fish size, individual growth, and energy acquisition, suggests that density dependency in Appalachian brook trout may be driven by periodic prey resource scarcity.

Résumé : On a identifié de l'agression, de l'émigration, de la mortalité et de la croissance individuelle dépendantes de la densité chez des populations sauvages de salmonidés d'eau courante. Cependant, la plupart des recherches se sont concentrées sur des poissons du genre *Salmo* et peu d'études ont tenu compte de la densité-dépendance en fonction de l'acquisition de l'énergie. Nous tentons de mesurer la croissance, la taille et l'acquisition de l'énergie en fonction de la densité dans une population d'ombles de fontaine (*Salvelinus fontinalis*) dans la région des Appalaches où l'espèce est limitée par la nourriture et où les densités de population sont basses. Nous avons suivi de très près des populations d'ombles de fontaine pendant 2 années à plusieurs sites dans un bassin versant de l'est de la Virgine Occidentale. Le marquage a fourni des estimations spécifiques de la croissance et des analyses du régime alimentaire ont donné des estimations de l'acquisition de l'énergie en fonction des besoins du maintien chez les poissons adultes. Les jeunes de l'année (YOY) présentent des relations de dépendance de la densité pour la taille, mais l'intensité de la densité-dépendance varie considérablement d'un mois à un autre et les pentes diminuent progressivement. La croissance individuelle et l'acquisition d'énergie excédentaire chez les adultes sont dépendantes de la densité, mais seulement durant les périodes de température élevée et d'ingestion faible d'énergie. La forte influence de la variabilité temporelle de la densité-dépendance sur la taille des YOY et sur la taille, la croissance individuelle et l'acquisition de l'énergie des adultes laisse croire que la densité-dépendance chez les ombles de fontaine des Appalaches peut s'expliquer par une rareté périodique des ressources en proies.

[Traduit par la Rédaction]

Introduction

Identifying density-dependent processes is a critical though often difficult step in assessing fish population dynamics. Density dependency occurs when intraspecific exploitation or interference competition increases with density, which can lead to elevated mortality rates, a greater propensity for individuals to emigrate, and (or) reduced growth rates (Rose et al. 2001). Such responses may be related to one another, as density-dependent individual growth may affect time spent susceptible to certain predators, thereby affecting mortality (Werner and Gilliam 1984). Despite the potentially important consequences, determining if density dependency is occurring can be difficult in wild populations. Strong temporal or spatial variability may mask density-dependent responses (Ray and Hastings 1996). Furthermore, density dependency may be temporally variable (Webster 2003), rendering sampling periodicity a critical factor.

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Density-dependent individual growth and size relationships in populations of lotic salmonids are commonly detected, though exceptions occur. Observational and experimental work has identified negative relationships between density and individual growth or size (regarded as a surrogate for growth), usually with fish in the genus *Salmo* (Vøllestad et al. 2002; Sundström et al. 2004; Vincenzi et al. 2007). The repeated occurrence of strong density-dependent individual growth and size relationships at low population densities has led to the suggestion that exploitative competition is the operating process, as space is unlikely limited at low densities (Jenkins et al. 1999; Post et al. 1999; Rincón and Lobón-Cerviá 2002). At high (≥2 individuals·m^{-2}) densities, however, space may become so limiting that decreasing individual growth occurs simultaneously with elevated mortality (Lobón-Cerviá 2007). Despite the growing body of evidence demonstrating density-dependent individual growth in lotic salmonids, studies reporting a lack of such relationships also exist (Rincón and Lobón-Cerviá 2002; Grant and Imre 2005).

Consideration of ontogeny may be crucial when assessing density dependency in lotic salmonids. Though some report that younger age classes exhibit greater density-dependent individual growth (Jenkins et al. 1999), several others have demonstrated the opposite scenario (Rincón and Lobón-Cerviá 2002; Arnekleiv et al. 2006; Einum et al. 2006). Energetic demand and territory size increase with increasing body size (Keeley and Grant 2001; Einum et al. 2006); therefore the idea that density-dependent individual growth increases as fish age is intuitive. However, the point at which density dependency begins to occur and the degree to which it is maintained in a cohort of lotic salmonids may be variable among populations, species, and environmental conditions.

Appalachian brook trout (*Salvelinus fontinalis*) represent a potentially informative case study to test the universality of density dependency in lotic salmonids. Glacial retreat since the Pleistocene confined most Appalachian brook trout populations to headwater watersheds, where streams are very low in productivity and often affected by acidic deposition. As a result, brook trout densities are typically low (<≈0.5 N·m^{-2} in some cases) relative to salmonid densities observed elsewhere. The age and size structure of Appalachian brook trout populations are also relatively unique. Though young-of-the-year (YOY) emerge in the spring and grow rapidly during their first growing season, adults seldom live beyond 2 years and rarely grow longer than 200 mm (Whitworth and Strange 1983). Such environmental and life history attributes are atypical of previous studies addressing salmonid density dependency.

We sought to determine the degree to which individual growth, energetic gain, and body size exhibit density dependency in Appalachian brook trout. The current study complements previous investigations on seasonal dynamics of prey importance and energetic gain (Utz and Hartman 2006, 2007; Utz et al. 2007). Specific questions that were addressed include the following: (*i*) how does individual growth vary throughout the year between age classes, and with respect to energy acquisition, (*ii*) is density-dependent individual growth and size exhibited in populations of Appalachian brook trout and, if so, do such relationships vary with seasonal change, and (*iii*) do density-dependent individual growth and size dynamics differ between adults and YOY?

Materials and methods

Site and brook trout population description

All sites were located in the Middle Fork River watershed within the MeadWestvaco Ecosystem Research Forest in Randolph County, West Virginia, USA. Land use in the watershed (and riparian areas of each study reach) consists of secondary-growth hardwood forest. Nine first- and second-order sites were selected to encompass a range of stream sizes and brook trout densities (Table 1); all supported YOY throughout the study. The close proximity (≤6 km distance between sites) likely rendered variation in flow regimes among sites negligible. Because of nearly identical climatic and riparian canopy conditions, mean temperature differences were <2 °C. No species of salmonid other than brook trout were collected over the course of the study.

The brook trout population in the Middle Fork River is typical of those in the Appalachians. YOY exhibit rapid growth rates during their first growing season, but growth slows thereafter; the mean size of individuals not deemed in the YOY cohort was 123.8 ± 1.2 mm (only 33 out of 3166 collected exceeded 200 mm in length). Although YOY are easily distinguishable from age-1+ fish using length–frequency histograms, delineation of adults to specific cohorts is impossible without use of scales or otoliths (not collected in the current study). In a separate study, age delineation using scales and otoliths within the Middle Fork River watershed suggested that 91% of age-1+ individuals consisted of 1- and 2-year-old trout; the remainder were age-3 (Stolarski 2007). All fish not considered YOY are hereafter referred to as adults.

Fish and habitat sampling

Fish were sampled 11 times in 2004 and 2005 (March, May, June, and July during both years and August, September, and December during 2004). The study design focused on consumption dynamics; thus warm seasons were overrepresented. At each site, a 200 m reach of stream was blocked with nets and triple-pass electrofished using DC current backpack units. All fish were immobilized with a dilute solution of clove oil and stream water; the fork length and weight of each fish were recorded. Ten fish representing the range of adult sizes at each site were subjected to gastric lavage for diet analysis during each sampling event. Adult fish were tagged with a unique identifier code in the caudal fin using visual implant elastomer tags (Northwest Marine Technology, Shaw Island, Washington) in May 2004 and 2005 (additional tagging was not performed because of the long handling time required). Habitat sampling was conducted during baseflow conditions in August 2005 and included measurements of wetted width (used to calculate fish density).

Consumption and maintenance ration

We estimated instantaneous energy available for growth as the difference between energy consumed and mainte-

Table 1. Study site attributes, Middle Fork River watershed, West Virginia.

Site	Strahler order	Basin area (km^2)	Mean riffle depth (cm)	Mean pool depth (cm)	Mean trout density ($N \cdot m^{-2}$)
Brush	1	0.83	7.58	19.30	0.115
Kittle, low	2	15.38	13.00	23.78	0.035
Kittle, mid	2	5.26	11.94	20.67	0.085
Kittle, up	1	2.05	7.46	17.54	0.237
Light	2	5.24	9.92	19.62	0.053
Mitchell	1	1.78	7.87	15.53	0.186
Rocky, low	2	9.94	15.21	30.83	0.028
Rocky, up	2	6.44	11.22	32.52	0.046
Sugar	1	1.64	8.09	27.92	0.151

Note: "Low", "mid", and "up" refer to the location of different sites on the same stream separated by ≥2 km.

nance ration for each fish and time interval. Estimated maintenance ration (EMR, in joules per gram of fish wet weight per day) was the consumption necessary to achieve zero growth in mass over 1 day. The EMRs were calculated based on a revised and validated bioenergetics model for brook trout (Hartman and Cox 2008) using fish weight, fish energy density, and water temperature as variables. Brook trout energy densities were calculated from mean energy density in collected fish using a Salmonidae dry weight – energy equation (Hartman and Brandt 1995). Consumption estimates were calculated based on the model proposed by Eggers (1977). Individual prey were converted to units of energy using taxon- and stage-specific dry weight – energy equations from Cummins and Wuycheck (1971), and the total energy in each gut were summed for each fish.

Daily consumption for each fish was calculated using the Eggers model, gut energy content, and temperature-specific gastric evacuation rates. The model is of the form

$$C = (GEC \cdot R)/W$$

where C is hourly consumption rate ($J \cdot g^{-1} \cdot h^{-1}$), GEC is gut energy content, R is the gut evacuation rate, and W is the weight of the fish. The hourly consumption rate is multiplied by 24 to convert consumption to a daily rate ($J \cdot g^{-1} \cdot day^{-1}$). Daily consumption estimates commonly use information on feeding intensity over a diel cycle. However, with knowledge of the diel feeding periodicity, or if there are no diel trends, single estimates of gut contents have been used successfully to estimate consumption in fish (Ruggerone 1989; Whitledge and Hayward 2000). We assumed that because several studies found no diel trends in feeding of brook trout (Forrester et al. 1994; Sweka 2003; Mookerji et al. 2004), daytime collections of gut contents were adequate to describe consumption rates and any errors would be systematic across sites, seasons, and densities.

The Eggers model requires an estimate of gastric evacuation rate (GER) to estimate consumption. Earlier estimates of consumption (Utz and Hartman 2006) used GERs published for brook trout by Sweka et al. (2004). GERs in fish are known to be influenced by fat and energy content of prey (Elliott 1972; Jobling 1987; Jensen and Berg 1993), prey size (He and Wurtsbaugh 1993), and meal size (Jobling et al. 1977), which is influenced by pre-experiment fasting periods (Bull and Metcalfe 1997). Methods used to estimate GERs by Sweka et al. (2004) may have been biased by long (5 days) pre-experiment fasting periods, as well as the use of fat, high-energy prey. To evaluate this, we compared the GER of Sweka et al. (2004) with other published salmonid exponential GERs (Fig. 1). We determined that other insect-feeding salmonids with shorter pre-experiment fasting periods had evacuation rates 3–8 times higher at a given temperature (Arctic char, *Salvelinus alpinus* (Amundsen and Klemetsen 1988); coho salmon, *Oncorhynchus kisutch* (Brodeur and Pearcy 1987); brown trout, *Salmo trutta* (Elliott 1972); rainbow trout, *Oncorhynchus mykiss* (Hayward and Weiland 1998); Chinook salmon, *Oncorhynchus tshawytscha* (Principe et al. 2007)) than those reported for brook trout. In addition to Sweka et al. (2004), Boisclair and Sirois (1993) published GER for brook trout feeding on *Artemia* that also showed slower GER than other salmonids. A study of the closely related Arctic char (Amundsen and Klemetsen 1988) found GER to fall in line with that of other salmonids feeding on natural insect or crustacean prey. Because food type, fat content, and fasting periods are such important influences on GER, we used previously published studies to develop a generalized salmonid GER model to predict evacuation rate of insect prey from temperature (analysis of variance, ANOVA, $df = 14$, $F = 38.53$, $p < 0.001$, $r^2 = 0.76$) and used this for calculating consumption of brook trout in this study (Fig. 1). The derived equation was

$$R = 0.0354 \cdot e^{0.1197T}$$

where R is the gastric evacuation rate (h^{-1}) and T is temperature (°C).

Statistical analyses

Population estimates of all fish were calculated for each stream using the Zippin model in program CAPTURE (White et al. 1982) to estimate fish density. If fewer than 30 fish were captured, then the actual number of fish caught was substituted for an estimate (Riley and Fausch 1992). Fish in the YOY cohort were easily identified using length–frequency histograms for collections from May through December. Although some YOY were collected as early as May, they did not fully recruit to the electrofishing gear (i.e., YOY counts were stable rather than increasing at each site) until July. Therefore potential density dependency of mean size in the YOY cohort was analyzed from July through December 2004 only.

Fish density per site was calculated as the population esti-

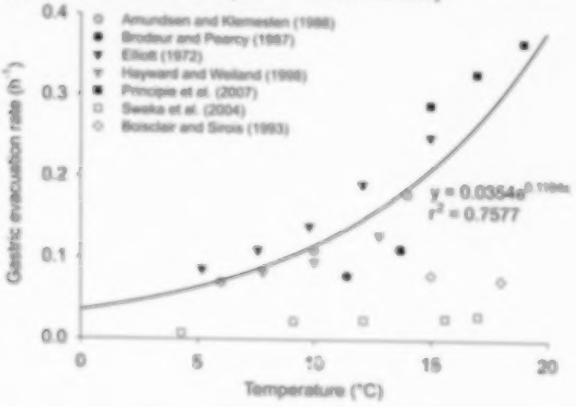


Fig. 1. Comparison of exponential gastric evacuation rates (GER) for salmonids from various studies. Solid and shaded symbols represent those studies used to derive the equation shown, which was applied to estimate consumption in the current study.

mate divided by the wetted area (m^2) during base flow. Early analyses considered the size-adjusted variable effective density (Σ(fish length)^2 divided by wetted area; Post et al. 1999) and YOY density as alternative predictor variables to the density of all trout. However, for our analyses, we considered only density for several reasons. Density was strongly correlated to both effective (Pearson's correlation coefficient = 0.96, $n = 9$, $p < 0.0001$; site-specific averages across all sampling events) and YOY density (Pearson's correlation coefficient = 0.96, $n = 9$, $p < 0.0001$; site-specific averages during sampling events when YOY were collected). Further, density consistently proved to be a superior predictor of response variables and represented the most parsimonious independent predictor among the density measures.

The size variable used in growth and size analyses was fork length due to the poor accuracy of weight estimates for small fish and the confounding effects of egg hydration on the weights of spawning females during fall. Growth (applied to recaptured fish) was calculated from the following equation (from Elliott 1975):

$$G_w = \ln(L_t/L_0)/\Delta t \times 100$$

where G_w is instantaneous growth (percentage of initial length gained between times 0 and t), L_t is the length at time t, L_0 is the initial length, and Δt is the amount of time elapsed between capture events (in days).

To assess density-dependent size relationships, a repeated-measures analysis of covariance (ANCOVA) was run on site-specific mean fish length for adults and YOY separately. We assumed that size would be temporally autocorrelated, as a proportion of the individuals collected would be recaptures but that the degree of autocorrelation would decay with time as fish either dispersed or grew. We therefore selected an autoregressive covariance structure. Initial model parameters included density, month, a month × density interaction term, and basin area. If basin area was not significantly related to mean fish size, it was removed and the models were reassessed. We considered both linear and power relationships between fish size and density. The model with the lowest AIC (Akaike's information criterion) value was selected. Following the model selection, mean fish size was regressed with mean density across all months (if the month × density term was not significant) or each month separately (if the month × density term was significant) to estimate the variation in mean size explained by density.

Potential density-dependent relationships in mean observed growth and mean excess energy acquisition were also explored. Each month was classified based on feeding success (from Utz and Hartman 2006) and temperature. Months fell into one of three seasons that were analyzed separately: favorable feeding conditions (May–June; four sampling events), poor feeding – warm (July–September; four sampling events), or poor feeding – cold (December–March; three sampling events). We were less concerned with temporal autocorrelation of variables for these analyses as we assumed that growth and energy acquisition were subject more to food availability than to resident fish characteristics. Therefore, for both individual growth rates and excess energy, the site-specific means were treated as response variables and site-specific mean density and basin area were treated as independent variables. To explore how trout density affected growth and excess energy acquisition, a multiple regression model was constructed with basin area and density as initial predictor variables. Basin area was removed if it proved to be unrelated to the dependent variable. Model AIC values were used to select linear versus power fits. All statistical procedures were performed in SAS (version 9.1, SAS Institute Inc. 2003).

Results

Variation in the growth rates of tagged fish reflected trends in excess energy acquisition (Fig. 2). Brook trout consumed the most excess energy and exhibited the largest individual growth rates during spring and early summer. Once energetic intake dropped in midsummer, growth fell to near zero and remained at low levels until the following spring. Trends in both growth and excess energy acquisition appeared to be consistent between 2004 and 2005, though the period of greatest excess energy intake (May 2004) did not directly correspond to the greatest observed growth rates. The most substantial deviance between the two variables occurred when the second greatest excess energy intake recorded (May 2005) did not correspond to the highest individual growth rates; this may have been an artifact of the poor recapture rate (13 fish). Unfortunately, fish tagging did not occur until May 2004; thus individual growth rates between March and May 2004 were not available.

Although mean fish length in the YOY age class was significantly related to trout density, temporal variation in the strength of density dependence was observed. Because month ($F = 123.5$, $p < 0.0001$), density ($F = 53.0$, $p < 0.0001$), and the density × month interaction term ($F = 14.1$, $p < 0.0001$) were significant in the selected model, mean length was regressed with density separately from July through December 2004. Density dependency in mean YOY size increased as the year progressed, with the most negative slope observed in December (Fig. 3). A linear relationship was superior to a power curve, whereas basin area was not significant. The repeated-measures approach proved

Fig. 2. Adult brook trout (*Salvelinus fontinalis*) mean growth rate (±95% confidence intervals; solid circles) and mean excess energy acquisition (estimated daily consumption – estimated maintenance ration; shaded bars) averaged across all sites. Month represents the ending month of the growth estimates. Numbers above error bars represent the sample size of recaptured tagged fish; each bar represents a sample size of 90 consumption estimates. March and May 2004 sampling events are not shown as fish were initially tagged in May 2004.

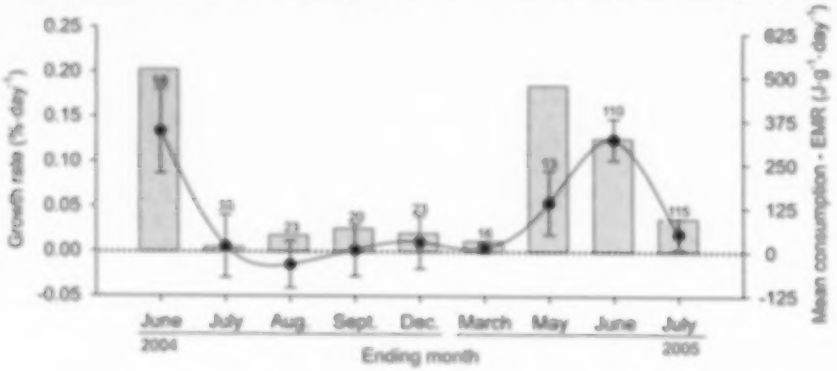


Fig. 3. Mean site-specific young-of-the-year (YOY) length as a function of trout density and sampling month (2004 only); β represents the slope of the linear relationship.

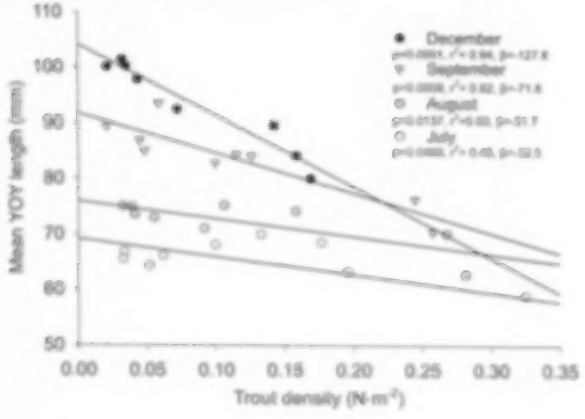
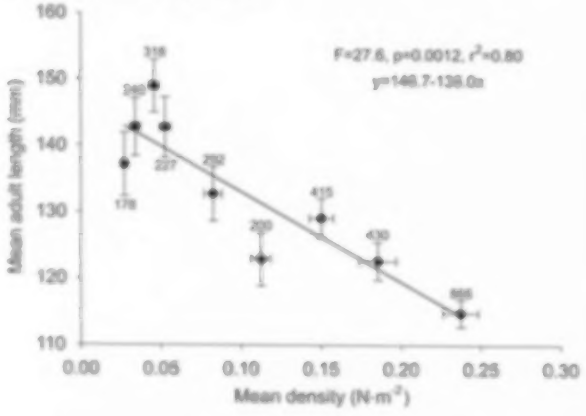


Fig. 4. Mean site-specific adult fish length as a function of mean trout density across all sampling events. Error bars represent 95% confidence intervals; numbers above or below the error bars represent site-specific sample sizes.



appropriate: the null model likelihood ratio test was significant at the level of $\alpha = 0.05$ ($\chi^2 = 9.08$, $p = 0.0026$)

Density dependency of mean length in the adult age class was also observed but appeared to be consistent through time. In the selected ANCOVA model, month ($F = 5.7$, $p < 0.0001$) and density ($F = 44.6$, $p < 0.0001$) were significant, whereas the interaction term was not ($F = 1.24$, $p = 0.2863$). As in the YOY cohort, a linear fit with density was found to be superior. When mean adult length was regressed with mean density across all sampling periods, a significant negative linear relationship was evident (Fig. 4).

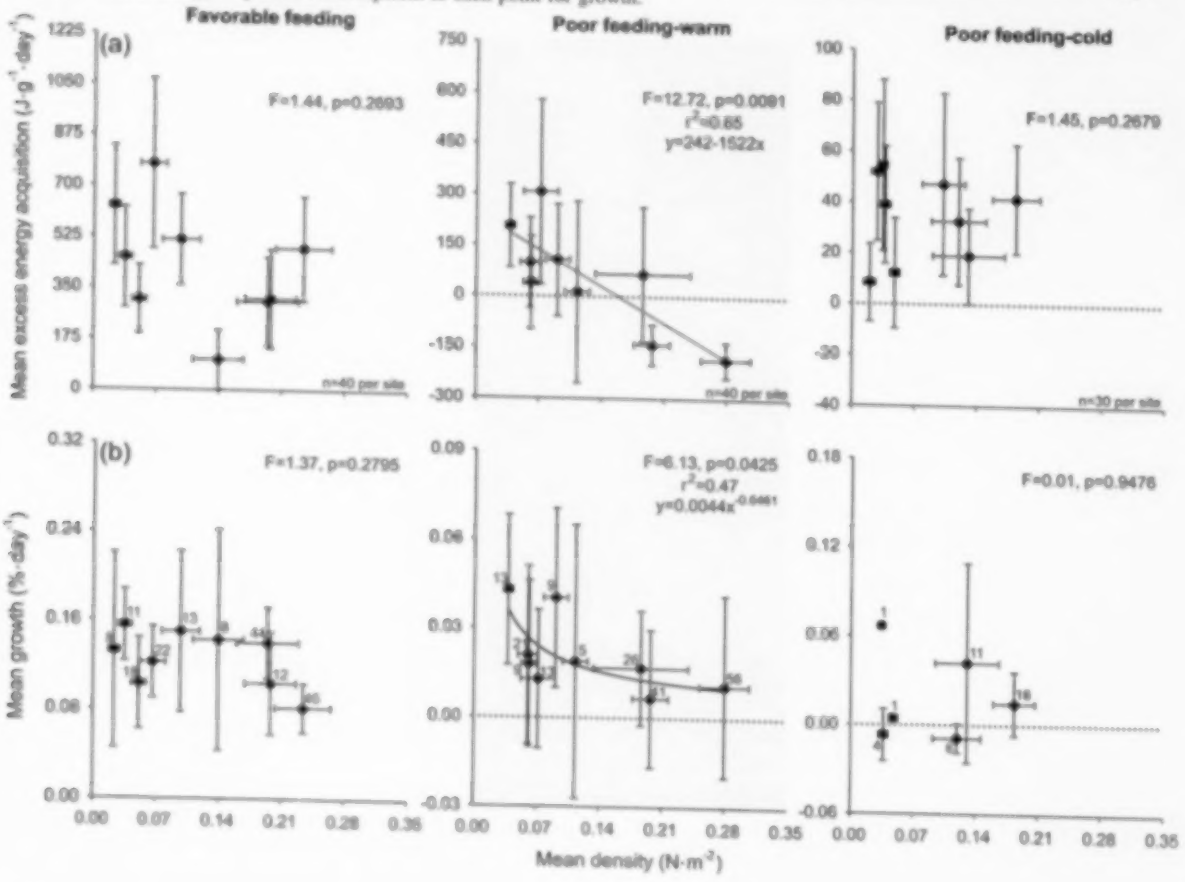
Mean excess energy acquisition was related to trout density, but only during the period of poor feeding and warm temperatures (July–September; Fig. 5*a*). During this season, a linear fit with density proved to be the best model and explained much of the variability in mean energy acquisition. In the remaining two seasons, however, no relationships with density were significant.

Patterns of density dependency in tagged fish growth reflected trends in excess energy acquisition. The only significant relationship was observed during the period of warm temperatures and poor feeding (Fig. 5*b*). Here, a power relationship proved to be the best model and explained approximately half the variation in mean growth. No significant relationships between mean trout density and mean growth were observed in the other seasons. Only six streams were regressed during the poor feeding – cold season because of poor recapture rates and absence of tagged fish in March 2004.

Discussion

Density-dependent growth and size relationships in Middle Fork River brook trout do exist but appear to be driven by energetic dynamics during metabolically stressful periods. From July through September, mean energy acquisition bordered on maintenance rations; fish in low-density streams fed above maintenance ration, whereas fish residing in high-density streams fed below. Consequences of density-dependent feeding were reflected in mean summer–fall in-

Fig. 5. (*a*) Mean site-specific excess energy acquisition (estimated daily consumption minus estimated maintenance ration) and (*b*) mean growth as functions of brook trout density. Three seasons were separately analyzed: spring ("Favorable feeding"; May–June), when temperatures were warm and consumption was high; summer ("Poor feeding – warm"; July–September), during high temperatures and consumption; and winter ("Poor feeding – cold"; December–March), when temperatures were cold and consumption was low. Solid lines denote significant relationships; error bars represent 95% confidence intervals. Site-specific sample sizes are provided in the lower right corner for excess energy acquisition and adjacent to each point for growth.



dividual growth, which likely led to the overall trends observed in density-dependent mean size between streams. High energy intake (assumed to be directly related to prey availability; Utz and Hartman 2006) appears to buffer density dependency during spring, whereas cold temperatures and reduced metabolic demand may render density dependency in winter months less significant during this period (though the small sample size of recaptures during winter likely disallows strong inferences on growth).

The observed trends in density-dependent individual growth of tagged adult fish are somewhat surprising in light of the size structure among sites. Both mean adult fish size and mean individual growth rates for recaptured fish were negatively related to density during the summer months. Larger fish, however, should be expected to naturally exhibit slower growth rates in terms of the proportion of initial length added over time because growth capacity declines with increasing size (Jobling 1983; Bjornsson and Tryggvadottir 1996). Results showing density-dependent individual growth in adult fish despite the disparity in mean size among sites imply that density was a strong driver of individual growth rates. The concordance between feeding intensity and growth estimates, however, offers a mechanistic explanation of the observed trends (prey scarcity as reflected by energy intake).

That density dependency may be driven by periodic prey resource scarcity may lend a partial explanation as to why reports of density dependency vary. Though significant density-dependent size and individual growth relationships appear to be increasingly consistent in the literature, exceptions are noted (Rincón and Lobón-Cerviá 2002; Grant and Imre 2005). Seasonal trends in energy acquisition demonstrated in the Middle Fork River (Utz and Hartman 2006) seem to be consistent for brook trout in the eastern United States (Ensign et al. 1990; Carlson et al. 2007). Substantial fluctuations in energy intake due to variable prey availability have been observed in populations of lotic salmonids elsewhere (Kawaguchi and Nakano 2001; Rundio and Lind-

ley 2008). Considering that most research addressing density dependency in lotic salmonids sample fish once within a year, previously reported results may have been partially affected by intra-annual variability in prey resources. Findings from this study warrant careful consideration of temporal variability in critical environmental factors (such as prey availability) when assessing density dependency.

YOY density-dependent size relationships also differed substantially among seasons. The weakest relationship between YOY size and density was observed in July, perhaps due to low absolute metabolic demand. However, as the year progressed and YOY fish grew, density dependency in mean size became stronger with time in both the slope of the relationship and the proportion of variance explained. The steepest slope appeared in December, after the period of time that most substantially affected the adult fish (summer–fall) had elapsed. Although others have shown increasing density-dependent consequences as fish age (Elliott and Hurley 1998; Rincón and Lobón-Cerviá 2002; Arnekleiv et al. 2006), the current analysis seems to be the first observational study to show such trends within a single growing season. In fact, the Middle Fork brook trout contrast trends demonstrated by Rincón and Lobón-Cerviá (2002) and Arnekleiv et al. (2006) in that density-dependent sizes were not observed for YOY cohorts in those systems. In a related manipulative experiment, Einum et al. (2006) report near identical findings to ours in populations of Atlantic salmon (*Salmo salar*) (compare our Fig. 3 with their fig. 1). Such trends have implications for other studies in which YOY density-dependent dynamics are examined: the choice of sampling period within the year may affect results.

Exploitation competition is the most commonly suggested driver behind density-dependent trends in lotic salmonids; our research lends cautious support to this notion. Prey resources, as suggested by excess energy acquisition, fluctuated substantially throughout the study. In the Middle Fork River watershed, density dependency in growth appeared in tagged fish only when prey resources were apparently low and temperatures were high, though our ability to make inferences during winter is limited due to poor recapture rates. Still, our findings suggest that exploitation competition is driving density dependency, which is often the conclusion drawn in related studies that did not quantify consumption or prey availability (Rincón and Lobón-Cerviá 2002; Imre et al. 2005; Arnekleiv et al. 2006). Yet exploitation and interference competition may not be mutually exclusive (Ward et al. 2007). Related studies (Rincón and Lobón-Cerviá 2002; Imre et al. 2005; Arnekleiv et al. 2006) suggest that fish densities similar to those observed in the Middle Fork River are too low to lend support for interference competition. This would seem a premature statement for the Middle Fork River population, as even in these low-density streams, ≥10 fish were retrieved from a single pool in some instances (R. Utz, personal observation). Therefore, interference competition should not be ruled out without concurrent examination of animal behavior in a given system.

Our findings contrast the few earlier examinations of density dependency in brook trout and may have management implications for the species. Previous studies in brook trout demonstrate either no density dependency in mean size (Hunt 1974, assessed in Imre et al. 2005; Dunham and Vinyard 1997) or weak density-dependent size relationships at much higher densities than those in the Middle Fork River watershed (Grant and Kramer 1990). High-density streams may be important source reaches for brook trout populations at the watershed scale (Petty et al. 2005). However, density-dependent growth in these systems, coupled with greater energy intake in lower reaches, suggests that low-density reaches are important for feeding and growth. Density dependency in YOY individual growth may also be critical for population regulation, as energy intake is strongly dependent on seasonally variable inputs of large terrestrial prey (Utz and Hartman 2007; Utz et al. 2007) only available to fish with a large enough gape.

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The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions

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Abstract: Nutrient diffusing substrata were used to determine the influence of inorganic nitrogen (N) and phosphorus (P) availability on community respiration (CR), gross primary production (GPP), and chlorophyll *a* (chl *a*) on inorganic and organic substrata. We incubated substrata in nine streams each in a total of eight ecoregions (n = 72 streams) located in a range of native vegetation, agriculture, and urban land-use types. On organic substrata, CR was nutrient-limited in 94% of reference streams but showed significant nutrient limitation in only 60% and 65% of agricultural and urban streams, respectively. The relative magnitude of nutrient limitation for CR on organic substrata decreased with increasing percent modified land use in the basin (agriculture + urban). On inorganic and organic substrata, GPP and chl *a* were rarely nutrient-limited across all ecoregions and land-use types, although the magnitude of nutrient limitation increased with increasing light availability. The effect of human land use on nutrient limitation of biofilm CR, GPP, and chl *a* was influenced by ecoregion, yet heterotrophic biofilms were consistently most sensitive to nutrient enrichment across ecoregions. Both heterotrophic and autotrophic biofilm constituents should be considered to fully understand stream ecosystem responses to nutrient enrichment.

Résumé : Nous avons utilisé des substrats diffuseurs de nutriments pour déterminer l'influence de la disponibilité de l'azote (N) et du phosphore (P) inorganiques sur la respiration de la communauté (CR), la production primaire brute (GPP) et la chlorophylle *a* (chl *a*) sur des substrats inorganiques et organiques. Nous avons incubé des substrats dans neufs cours d'eau dans chacune d'un ensemble de huit écorégions (n = 72 cours d'eau) situés dans une gamme de végétations indigènes, de types d'agriculture et d'utilisatiosn urbaines des terres. Sur les substrats organiques, CR est limitée par les nutriments dans 94 % des cours d'eau témoins, mais elle ne montre de limitation significative par les nutriments que dans respectivement 60 % et 65 % des cours d'eau agricoles et urbains. L'importance relative de la limitation de CR par les nutriments sur les substrats organiques diminue en fonction de l'augmentation du pourcentage d'utilisation de terres modifiées (agriculture + urbanisation) dans le bassin versant. Sur les substrats inorganiques et organiques, GPP et chl *a* sont rarement limitées par les nutriments sur l'ensemble des écorégions et dans les différents types d'utilisation des terres, bien que l'importance de la limitation par les nutriments augmente en fonction de la disponibilité de la lumière. L'effet de l'utilisation des terres par les humains sur la limitation par les nutriments de CR, GPP et chl *a* de biofilms varie selon l'écorégion; néanmoins, les biofilms hétérotrophes sont régulièrement plus sensibles à un enrichissement en nutriments dans toutes les écorégions. Il faut tenir des constituants hétérotrophes et autotrophes des biofilms si l'on veut comprendre entièrement la réaction des écosystèmes d'eau courante à l'enrichissement en nutriments.

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Introduction

Benthic biofilms in streams influence ecosystem function via their role in primary production, heterotrophic production, nutrient spiraling, and organic matter decomposition (Cummins 1974). Autotrophic constituents of biofilms fix carbon (C) from the atmosphere, whereas heterotrophs use organic carbon for biosynthesis and growth (Schlesinger 1997). Although biofilm autotrophs and heterotrophs play unique roles in streams, their growth is often regulated by similar factors, including susceptibility to scour from flooding (Biggs and Close 1989; Ryder et al. 2006), temperature (Suberkropp and Chauvet 1995; Francoeur et al. 1999), and nutrient availability (Borchardt 1996; Gulis et al. 2004). Autotrophs are also influenced by light availability (Hill 1996) and heterotrophs can be regulated by substrate (i.e., carbon) quality (Peterson et al. 1993, Gessner and Chauvet 1994).

Nitrogen (N) or phosphorus (P) availability may limit growth of both heterotrophs and autotrophs (Pringle et al. 1986; Tank and Webster 1998). Biofilm autotrophs can be

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N-limited, P-limited, co-limited by N and P, or not limited by nutrients at all (Tank and Webster 1998; Francoeur 2001; Tank and Dodds 2003), and heterotrophs have an equally wide range of responses to nutrient amendment (Hepinstall and Fuller 1994; Tank and Webster 1998; Tank and Dodds 2003). Most importantly, autotrophs and heterotrophs often have different nutrient limitation status when directly compared with each other in the same system (Tank and Dodds 2003), which may reflect physiological differences in nutrient demand. Therefore, both autotrophic and heterotrophic biofilms are potentially sensitive to nutrient availability, and conditions that affect nutrient availability could have a strong and differential effect on nutrient limitation of autotrophic versus heterotrophic biofilms.

Human land use often leads to elevated nutrient concentrations (Carpenter et al. 1998; Kemp and Dodds 2001; Walsh et al. 2005). Agriculture increases nitrate (NO_3^-) concentrations as a result of excess fertilizer application (Howarth et al. 1996; Royer et al. 2004). Urban land use tends to increase ammonium (NH_4^+) and dissolved P concentrations from sewage and septic inputs and lawn fertilizer applications (Paul and Meyer 2001; Walsh et al. 2005). The type and intensity of land use within broad categories (e.g., agriculture, urbanization) is variable and therefore so is its effect on stream ecosystems (Allan 2004). For example, row-crop agriculture can result in extensive channel modifications and nutrient enrichment of stream ecosystems, whereas low-intensity agricultural livestock production in pastures can have a much less pronounced effect (Strayer et al. 2003; Allan 2004). Nutrient input to urbanized streams is generally correlated with population density and impervious surface area (Paul and Meyer 2001; Meyer et al. 2005; Walsh et al. 2005), which are both factors that vary dramatically across the landscape. We expect that autotrophic and heterotrophic nutrient limitation will also vary based on the intensity of human land use.

Variation in nutrient availability can also be driven by regional differences in climate, physiography, and vegetative cover (i.e., ecoregions; Bailey 1976; Omernik 1977), potentially translating into variation in nutrient limitation (Smith et al. 2003). For example, southwestern US streams frequently have low inorganic N concentrations and are subsequently N-limited (Grimm and Fisher 1986). Furthermore, Tank and Dodds (2003) found that biofilm nutrient limitation varied across ecoregions. Regional differences in background nutrient concentrations influence stream ecosystem management (Dodds and Welch 2000; Smith et al. 2003; Suplee et al. 2007). However, we are not aware of studies that have analyzed ecoregions and human land use effects on nutrient limitation across a broad geographic scale.

Our goal was to compare nutrient limitation of heterotrophic and autotrophic stream biofilms among a wide range and intensity of human land use, as well as among ecoregions of North America. Using nutrient diffusing substrata, we quantified N and P limitation across eight ecoregions in nine streams per ecoregion, three from one of each adjacent land-use type: native vegetation (or reference), agriculture, and urban (n = 72 streams total). We compared nutrient limitation of autotrophic and heterotrophic biofilms on two different substratum types (inorganic vs. organic), which served as surrogates for equivalent stream substrata such as cobble (inorganic) and leaves and wood (organic). We predicted that biofilm nutrient limitation in reference streams would differ based on ecoregion, substratum type, and biofilm type, and across all ecoregions, the intensity of human land use would influence autotrophic and heterotrophic biofilm nutrient limitation. These data from 72 streams greatly expand our understanding of how land use may influence biofilm nutrient use and, by extension, factors controlling stream ecosystem function across different ecoregions.

Materials and methods

Sites

Nutrient limitation was assessed via nutrient diffusing substrata (NDS) in 72 streams as part of the Lotic Intersite Nitrogen eXperiment II (LINXII), a large collaborative project examining the influence of land use on stream NO_3^- cycling. Nine headwater streams were selected in eight ecoregions across the US and Puerto Rico encompassing a wide range of climatic conditions (Table 1; for site map, also see Mulholland et al. 2008). For this study, ecoregions were abbreviated in the results using two-letter state postal codes except for the southwest (SW), which included streams in Arizona and New Mexico.

In each ecoregion, three streams were selected to span a range of three land-use types: native vegetation (or reference), agriculture, and urban. Land use was broadly categorized and initially based on visual observations of the riparian land use immediately adjacent to the study reaches (Table 1). Reference streams were bordered by native vegetation of the specific ecoregion including forest, prairie, and desert shrub land, with a coverage in the basin generally >85% (except two streams with 50% and 65%). Agricultural land use in stream basins was highly variable, ranging from row-crop agriculture with associated high fertilizer application to pastures with low-intensity cattle grazing, with a range in basin coverage from 4% to 100%. Urban land use was also variable, consisting of highly urbanized streams with cemented channels, residential–suburban streams, parkland, and even recreational (e.g., golf course) land use, with a range in coverage from ~1% to 100%. After site selection, the percent land cover of each land-use type for each basin was determined using the US Geological Survey (USGS) National Elevation Data Set and the 2001 USGS National Land Cover Datasets and for PR using the 1991–1992 Landsat TM imagery as derived by Helmer et al. (2002). Streams within each land-use category in each region were not considered replicates, but instead span a typical range representative of that land-use type in the ecoregion. Additionally, selection of agricultural and urban streams was not necessarily intended to represent dominance of that land-use type within the basin, and many basins consisted of mixed land uses. As part of the larger LINXII study, three streams (one from each land-use type) were typically studied in each of the eight ecoregions per year (n = 27 per year) for a total of three years (2003–2005, n = 72 streams in total) during base-flow conditions.

Nutrient diffusing substrata

NDS were constructed using 30 mL plastic cups filled with a 2% agar solution amended with 0.5 mol·L^{-1} $NaNO_3$ (N treatment), 0.5 mol·L^{-1} KH_2PO_4 (P treatment), both (N + P treatment), or no amendment as a control (C treatment; Tank et al. 2006). NDS were topped with either inorganic fritted glass disks or organic cellulose sponge cloth totaling five replicates for each treatment for each substratum type. Inorganic and organic substrata were chosen to examine nutrient limitation of a typical range in types of biofilms found in natural stream ecosystems. NDS were placed in riffles that were located in reaches representative of ambient light and flow conditions in each stream (as reported in Table 1). For example, if a stream was mostly shaded, NDS were placed in a shaded location, if a stream was open, then open conditions were sought for NDS placement. NDS were deployed in each stream for 17 days to ensure that nutrients continued to diffuse from the NDS throughout the entire incubation period (Tank et al. 2006). Prior work demonstrated that 17 days was ample time for development of a significant biofilm response in reference streams. NDS were then retrieved from streams, and substrata were removed from plastic cups and immediately placed in 50 mL centrifuge tubes filled with stream water. After transport to the laboratory, substrata were analyzed for autotrophic activity by measuring gross primary production (GPP) via dissolved oxygen (DO) production, autotrophic biomass using chlorophyll *a* (chl *a*) extraction, and heterotrophic community respiration (CR) via dissolved oxygen (DO) consumption (see methods described below).

In 2003, we quantified chl *a* on inorganic substrata and CR on organic substrata. We expanded the methods in 2004 and 2005 to include GPP and measured all three metrics (GPP, chl *a*, and CR) on both substrata, allowing us to confirm selection of autotrophic and heterotrophic biofilms by inorganic and organic substrata, respectively. We measured GPP along with chl *a* as indicators of autotrophic nutrient limitation. GPP may indicate more directly the limitation of ecosystem function, because it is a measure of autotrophic activity and state rather than solely autotrophic biomass (chl *a*), which can vary based on parameters other than nutrients (e.g., grazing, light availability).

GPP and CR were measured using a modified light–dark bottle method in which net community metabolism (NCM) was measured in the light, CR was measured in the dark, and GPP was calculated by adding NCM and CR (American Public Health Association (APHA) 1995; Hill et al. 2002). Centrifuge tubes containing substrata retrieved from NDS were refilled with fresh ambient water from each stream taking care to exclude all air bubbles from tubes. First, NCM was measured as the difference in DO after a 3 h incubation on a shaker table under a wide-spectrum fluorescent grow lamp (1900 lm, photosynthetically active radiation (PAR) = 35 μmol·m^{-2}·s^{-1}; model GE F40PL/AQ, The General Electric Company, Fairfield, Connecticut). We measured NCM with a grow lamp to maintain similar and consistent light conditions across all samples to compare nutrient limitation across biofilms from a variety of streams. Given potential differences in the response of GPP to the constant PAR conditions used during the incubations, we also calculated quantum yield (mol O_2·mol quanta^{-1}) by dividing GPP by incubation PAR to more accurately examine relationships with in situ light availability. Because the experimental PAR level was unlikely to be saturating (Dodds et al. 1999), this strategy allowed for comparison of photosynthetic efficiency independent of variation of in situ light across measurements.

Then, CR was measured using the consumption of DO after a 2 h incubation in the dark with gentle continuous mixing on a shaker table. The same substratum was used for both NCM and CR measurements, but fresh stream water was used for each analysis to maintain ambient nutrient availability and a starting point close to saturating DO concentrations. Following metabolism estimates, chl *a* was extracted from the same substratum using the hot ethanol method (Sartory and Grobbelaar 1984). Substrata were extracted in 95% ethanol, heated to 79 °C for 5 min, and then cooled for 24 h at 4 °C. Following centrifugation, chl *a* in the extract was measured using the nonacidification method on a fluorometer set to avoid interference from phaeophytin (Welschmeyer 1994).

Numerous descriptor variables were quantified in each stream as part of the larger LINXII experimental protocol, which occurred at or near the time of NDS placement, confirming that streams both within and across ecoregions were diverse (Table 1; Mulholland et al. 2008). NO_3^- concentrations were measured using ion chromatography or colorimetry, NH_4^+ concentrations were measured using indophenol colorimetry or fluorometry, and soluble reactive phosphorus (SRP) concentrations were measured using molybdate-blue colorimetry (APHA 1995; Holmes et al. 1999; Taylor et al. 2007). Light availability as percent channel shade was measured at each stream using a canopy densiometer, and PAR was measured every minute for three representative days of the experiment.

Calculations and statistical analyses

Nutrient limitation status (a categorical designation) for each response metric (GPP, CR, or chl *a*) on each substratum type (inorganic or organic) was determined using a two-way analysis of variance (ANOVA) based on the presence or absence of N and (or) P in the agar. For this ANOVA, we used N and P as the main factors, and significant interaction terms determined instances of co-limitation or secondary limitation by N and P (interpreted as in Tank and Dodds 2003). In addition to determining if there was significant nutrient limitation by N or P, we calculated the nutrient response ratio (NRR) as the ratio of the N + P treatment over the control treatment for CR on organic substrata and GPP and chl *a* on inorganic substrata (Francoeur 2001; Tank and Dodds 2003). Thus, a NRR >1 would indicate a positive response of either GPP or CR to added nutrients (N + P treatment > control). Using NRR in addition to nutrient limitation status allowed us to compare the magnitude of nutrient limitation on a relative scale across streams and ecoregions and relate this to other predictor variables used as ecoregion and stream descriptors (see Table 1; Mulholland et al. 2008).

We used a one-way analysis of covariance (ANCOVA) to examine the effect of ecoregion on stream characteristics and NRR for all response metrics with percent modified land use (percent agriculture + urban) in the catchment as the covariate followed by Tukey's post-hoc comparisons to

Table 1. Study stream locations, land-use classifications, and physical and chemical characteristics.

Site	Ecoregion (Omernik 1987)	Stream name	Land-use classification	Catchment area (km^2)	Discharge ($L \cdot s^{-1}$)	NO_3^- ($\mu g\ N \cdot L^{-1}$)	NH_4^+ ($\mu g\ N \cdot L^{-1}$)	SRP ($\mu g\ P \cdot L^{-1}$)	PAR (mol quanta $\cdot m^{-2} \cdot day^{-1}$)	Percent channel shade
MA	Eastern temperate forest: Northeastern Coastal Zone	Cart Creek	REF	3.9	4.8	15.3	293.2	1.5	1.2	76
		Boxford	REF	1.6	12.2	52.8	13.3	9.2	0.5	86
		Gravelly Brook	REF	5.6	2.0	112.2	435.4	80.3	0.7	7
		Runaway Brook	AGR	0.4	0.7	1 164.2	80.2	7.8	19	0
		Long Meadow Brook	AGR	1.0	2.4	989.3	63.1	10.8	4.5	26
		Black Brook	AGR	6.1	120.1	50.3	30.5	33.5	19.9	20
		IS_104	URB	1.2	2.1	1 336.2	121.2	2.1	1.2	91
		Sawmill Brook	URB	4.2	4.9	1 024.7	39.2	11.5	0.2	85
		IS_118	URB	2.1	11.3	512.8	253.8	11.8	9.7	65
NC	Eastern temperate forest: Blue Ridge Mountains	Hugh White Creek	REF	0.3	19.4	7.3	3.2	2.8	0.2	93
		Big Hurricane Branch	REF	0.6	12.2	240.7	5.6	2.5	1.7	93
		Cunningham Creek	REF	1.1	49.3	10.1	2.7	1.9	2.9	94
		Hoglot Branch	AGR	2.7	52.7	154.5	17.1	2.8	25.8	41
		Jerry Branch	AGR	2.6	26.5	405.7	108.2	18.2	6.9	41
		Blacks Branch	AGR	3.6	189.4	172.6	8.5	6.7	29.6	ND
		Crawford Branch	URB	3.0	45.0	102.5	15.4	4.3	2.3	90
		Mud Creek	URB	4.8	51.8	139.5	6.0	2.1	39.4	57
		Sugarloaf Creek	URB	3.7	79.8	54.2	2.6	2.9	ND	96
MI	Eastern temperate forest: Southern Michigan – Northern Indiana Till Plains	Sand Creek	REF	1.1	4.9	282.6	54.8	14.9	1.5	81
		Bullet	REF	3.6	6.5	384.5	11.0	2.3	9.2	42
		Honeysuckle	REF	5.1	99.4	4.2	21.1	3.7	30.3	91
		Steinke Drain	AGR	3.0	1.7	4 158.3	29.4	68.1	21.5	ND
		Buskirk	AGR	2.8	6.0	81.5	20.7	11.3	37.6	65
		Bellingham	AGR	2.9	22.9	1452.9	27.9	1.7	20.9	8
		Dorr	URB	1.9	35.0	1 100.2	127.7	9.2	11.1	ND
		Wayland	URB	2.7	11.7	694.6	74.3	5.4	ND	52
		Arcadia	URB	32.6	110.1	273.5	32.0	10.7	51	0
KS	Great plains: Flint Hills	Kings Creek N4D	REF	1.6	13.4	8.6	0.3	0.5	32.1	39
		K2A	REF	3.3	26.3	0.9	6.7	1.9	22.7	37
		Shane Creek	REF	4.4	4.4	1.2	4.7	1.0	51.7	57
		Agnorth	AGR	5.0	0.2	34.8	31.7	0.2	56	15
		Natalie Creek	AGR	0.9	1.3	6.0	3.1	2.4	29.5	44
		Swine	AGR	10.1	5.4	21 162.3	3.4	16.2	58.8	11
		Campus Creek	URB	1.7	2.9	2 942.0	7.8	4.0	49.8	71
		Walmart Ditch	URB	7.2	1.6	277.4	28.3	35.4	56.1	0
		Little Kitten	URB	5.6	20.1	167.6	24.2	7.2	52.4	76

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Table 1 (*concluded*).

Site	Ecoregion (Omernik 1987)	Stream name	Land-use classification	Catchment area (km^2)	Discharge ($L \cdot s^{-1}$)	NO_3^- ($\mu g\ N \cdot L^{-1}$)	NH_4^+ ($\mu g\ N \cdot L^{-1}$)	SRP ($\mu g\ P \cdot L^{-1}$)	PAR (mol quanta $\cdot m^{-2} \cdot day^{-1}$)	Percent channel shade
WY	North American deserts: Middle Rockies or Wyoming Basin	Ditch	REF	74.0	55.7	0.1	1.7	2.4	ND	19
		Two Oceans	REF	29.7	64.5	18.9	3.8	10.0	ND	3
		Spread	REF	260.4	267.8	2.8	2.2	ND	56.7	17
		Giltner	AGR	1.2	158.5	49.7	3.0	2.8	ND	0
		Headquarters	AGR	1.3	131.1	0.7	2.9	15.3	69.9	1
		Kimball	AGR	103.0	153.8	27.9	1.1	4.4	55.9	0
		Golf	URB	1 605.7	110.0	0.8	1.0	2.1	ND	39
		Teton Pines	URB	0.03	9.5	152.1	1.0	3.2	25.3	39
		Fish	URB	8.8	102.9	234.7	4.3	5.9	60.9	0
SW	North American deserts: Arizona – New Mexico Plateau	Agua Fria	REF	645.5	11.9	0.4	1.5	55.9	69.9	13
		Rio Salado	REF	3 410.8	5.8	3.8	3.6	2.5	56	0
		Sycamore Ck	REF	230.8	21.3	57.5	2.0	31.8	57.8	ND
		Bernalillo drain	AGR	0.8	23.5	1.5	1.8	36.7	69.2	0
		Rio Puerco	AGR	16 019.7	2.5	3.6	3.9	13.6	47.6	ND
		San Pedro	AGR	92.5	4.0	297.2	4.4	18.9	65.2	0
		Rio Rancho	URB	2.8	17.8	12.5	3.4	50.2	61.7	1
		Indian Bend Wash	URB	453.8	28.4	99.2	65.2	20.9	2.9	ND
		Tempe Town lake	URB	889.4	18.0	4.3	9.8	25.2	52.2	0
OR	Northwestern forested mountains: Cascades or Willamette Valley	Oak	REF	6.2	7.5	70.5	1.3	34.7	1.4	92
		Mack	REF	5.3	30.7	62.7	5.9	13.0	2	56
		Potts	REF	3.5	19.0	69.3	4.0	24.5	4	88
		Oak	AGR	30.5	5.5	96.0	8.4	47.8	2.7	75
		Camp	AGR	26.8	113.4	54.2	6.1	5.3	21.3	69
		Courtney	AGR	41.7	34.7	96.6	10.6	5.0	26.4	27
		Oak	URB	32.2	5.6	162.7	19.3	45.3	2.6	82
		Amazon	URB	21.8	25.0	2.4	4.8	17.8	39.4	49
		Periwinkle	URB	10.3	2.7	7.7	4.4	208.5	37.6	1
PR	Tropical wet forest	Bisley	REF	0.8	12.5	170.7	2.6	21.5	0.2	88
		RIT	REF	0.3	20.0	130.8	7.2	0.2	0.5	17
		Q. Pared	REF	0.8	5.2	104.7	2.5	7.3	1.3	57
		Grande	AGR	0.7	12.3	275.8	11.1	13.2	6.4	24
		Maizales	AGR	2.6	25.0	205.6	7.1	11.5	12.4	10
		Q. Vaca	AGR	1.7	111.9	445.7	2.6	8.7	1.3	57
		Petunia	URB	1.4	4.7	997.1	15.1	26.3	0.03	89
		Mtrib	URB	1.3	23.2	174.3	2204.3	310.5	0.7	65
		Q. Ceiba	URB	5.2	49.5	511.7	50.3	21.5	1.6	50

Note: Physical characteristics and photosynthetically active radiation (PAR) were calculated during the 24 h ^{15}N tracer experiment, and chemical characteristics were the mean from sampling dates up to a week after the experiment. SRP, soluble reactive phosphorus; ND, no data were available.

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examine specific pairwise differences in ecoregion. We also used simple linear regression (SLR) to identify controls on NRR. Paired *t* tests were used to examine differences in CR, GPP, and chl *a* between inorganic and organic substrata. Statistical analyses were performed using SYSTAT 11 (Systat Software, Richmond, California), and statistical significance was determined at the $\alpha = 0.05$ level. To meet the assumptions of parametric statistics, we tested the data using a KS–Lilliefors test for normality ($p > 0.05$) and then transformed non-normal data using logarithmic (CR, all NRR, QY, and NH_4^+ concentrations), arcsine square root (percent channel shading, percent modified land use), or power (GPP, chl *a*, PAR) transformations.

Results

Site characteristics

Across ecoregions and land use, our streams encompassed a wide range in water chemistry characteristics (NO_3^-, NH_4^+, and SRP concentrations) and light availability (PAR and percent channel shade; Table 1). NO_3^- concentrations ranged from 0.1 to 21 162 μg N·L^{-1}, with significantly higher concentrations in PR compared with the SW and WY ecoregions (one-way ANCOVA, $p < 0.001$). Additionally, NO_3^- concentrations significantly increased with increasing percent modified land use in the stream basin (one-way ANCOVA, $p < 0.001$), but the strength of this relationship depended on ecoregion (one-way ANCOVA, interaction $p < 0.001$). Similarly, NH_4^+ (range 0.3–2204 μg N·L^{-1}) and SRP (range 0.2–311 μg P·L^{-1}) concentrations increased with greater percent modified land use (one-way ANCOVA, NH_4^+ $p = 0.003$, SRP $p = 0.007$), but there were no significant differences by ecoregion (one-way ANCOVA, $p > 0.05$). Channel shade and PAR also differed by ecoregion (one-way ANCOVA, $p < 0.001$), with higher PAR in KS, SW, and WY compared with MA and PR and lower percent channel shade in SW and WY compared with OR, PR, and NC. Additionally, percent channel shade decreased with increasing percent modified land use (one-way ANCOVA, $p = 0.02$), but we found no relationship between PAR and percent modified land use (one-way ANCOVA, $p > 0.05$).

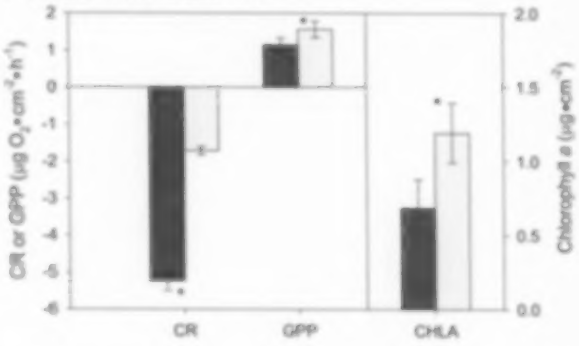
Selection of autotrophs and heterotrophs by substratum type

When combining NDS data across all nutrient treatments for each of our three response metrics (CR, GPP, and chl *a*), we found differences in biofilm function by substratum type (Fig. 1). CR was significantly higher on organic substrata compared with inorganic substrata (paired *t* test, $p < 0.001$), and the absolute value of CR was greater than GPP. In contrast, on inorganic substrata, GPP and chl *a* (autotrophic biomass) were significantly higher than on organic substrata (paired *t* test, GPP $p = 0.028$, chl *a* $p < 0.001$), and the absolute value of CR was approximately equal to GPP. Based on our response metrics (GPP and CR), organic substrata tended to be more heterotrophic than inorganic substrata, which tended to be more autotrophic.

Nutrient limitation of community respiration

Heterotrophic activity (CR) on organic substrata across all land-use types was frequently nutrient-limited, but there was no prevalence of N versus P limitation (Fig. 2). Considering reference streams alone, CR was most frequently N-limited, and when combined with P limitation and co-limitation by N + P, CR was nutrient-limited in all but one stream. In contrast to reference streams, CR was not nutrient-limited in a majority of agricultural and urban streams. Interestingly, the urban streams that were nutrient-limited showed predominantly P limitation, and more urban streams exhibited P limitation than reference streams.

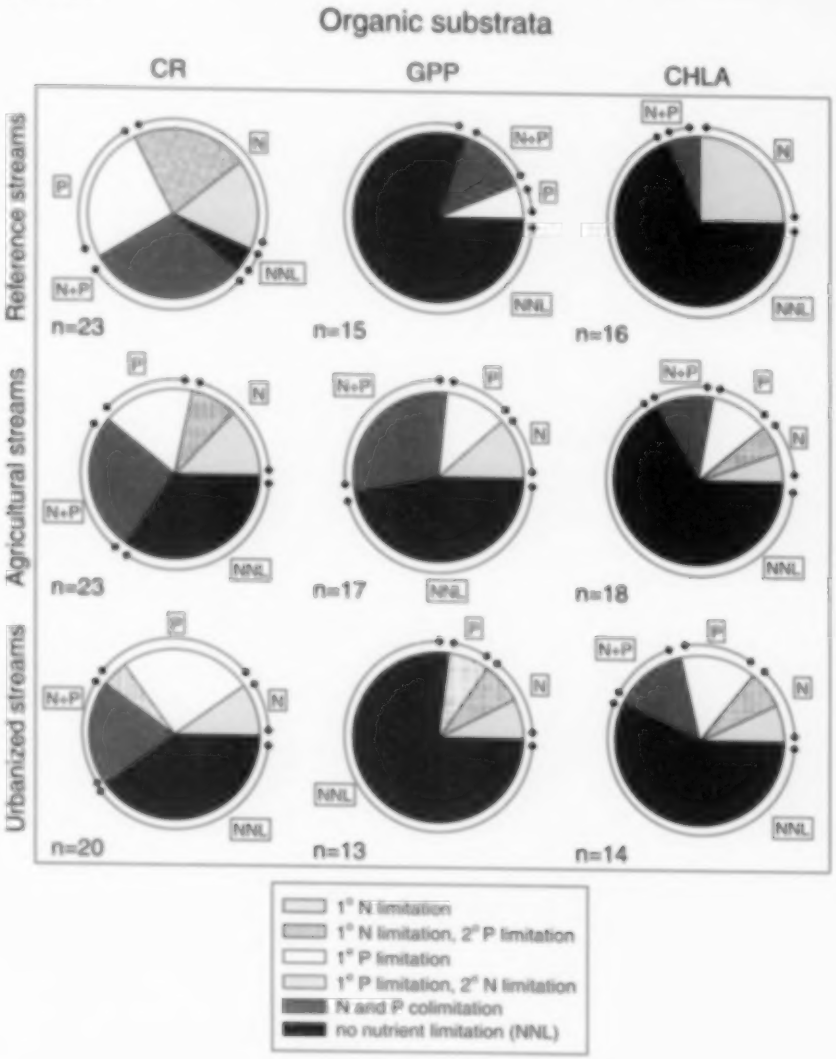
Fig. 1. The effect of organic (bars with dark shading) and inorganic (bars with light shading) substrata on community respiration (CR), gross primary production (GPP), and chlorophyll *a* (CHLA). Means ± standard error (SE) are reported. Asterisks (*) above bars represent significant differences ($p < 0.05$) between inorganic and organic substrata as determined by paired *t* test on transformed data. *P* values, data transformations, and *n* for each response were as follows: CR, $p < 0.001$, $\log(x + 1)$, $n_{organic} = 68$, $n_{inorganic} = 50$; GPP, $p = 0.028$, $x^{1/3}$, $n_{organic} = 46$, $n_{inorganic} = 50$; CHLA, $p < 0.001$, $x^{1/3}$, $n_{organic} = 49$, $n_{inorganic} = 72$.



Because CR was significantly higher on organic substrata than on inorganic substrata (Fig. 1), we examined factors that influenced the nutrient response ratio (NRR) for CR on organic substrata. Within ecoregions and pooling data across land-use types, the largest response to N + P addition for CR on organic substrata was in WY, the lowest was in KS and MA, and the remaining ecoregions had similar NRRs (Fig. 3*a*; one-way ANCOVA, $p = 0.001$). By pooling data across ecoregions, we found that NRR decreased as the percent modified land use (percent agriculture + percent urban) in the catchment increased; however, little variation in NRR was explained by percent modified land use ($r^2 = 0.143$; Table 2). Upon further investigation, we found differential effects of land use depending on ecoregion (one-way ANCOVA, $p = 0.011$). For example, there was a strong effect of land use on NRR (>45% decline in NRR from reference to agriculture or urban) in KS and NC, but only a moderate effect (10%–45% NRR decline) in MI and WY and only a weak effect (<10% NRR decline; Table 3) in MA and PR. Finally, for CR on organic substrata, we analyzed drivers of NRR and found that CR was relatively less nutrient-limited as stream water ammonium (NH_4^+) availability increased (Table 2).

CR was much less frequently nutrient-limited on inorganic substrata than on organic and was not nutrient-limited in 83% of all streams (Fig. 4). CR may have been limited by carbon availability across all streams because inorganic sub-

Fig. 2. Nutrient limitation status of community respiration (CR), gross primary production (GPP), and chlorophyll *a* (CHLA) on organic substrata in reference (native vegetation), agricultural, and urbanized streams. Primary (1°) and secondary (2°) nutrient limitation status, as defined in the key, was determined by a significant response to nutrient treatment: N alone, P alone, and N and P added together (analysis of variance, ANOVA, $p < 0.05$).

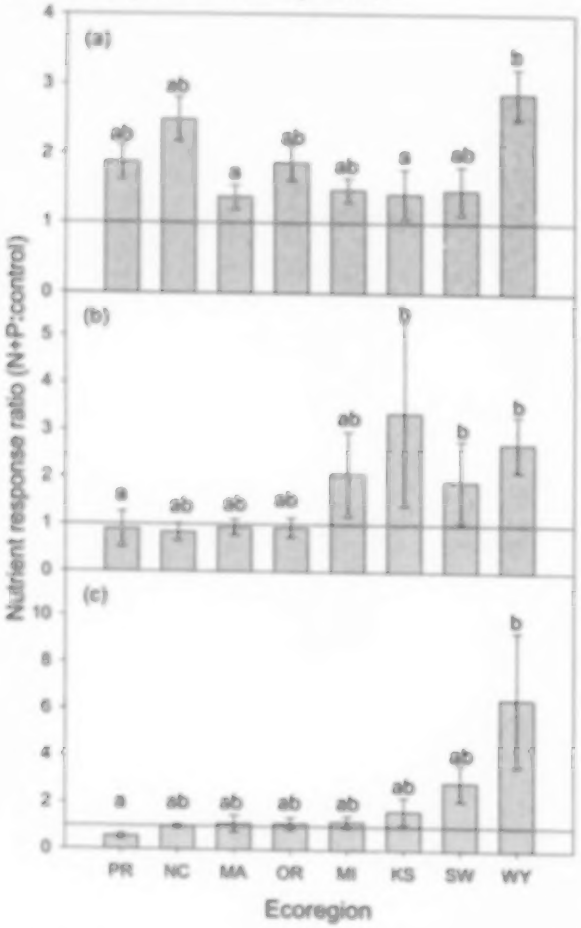


strata did not provide an outside source of carbon to heterotrophs. In the infrequent cases of nutrient limitation, reference streams were never P-limited and urban streams were never N-limited, whereas agricultural streams had cases of N limitation, P limitation, and co-limitation of N + P. Overall, even with land use, CR on inorganic substrata was rarely limited by N or P.

Nutrient limitation of gross primary production

In general, across all land-use types, we found that GPP on organic substrata was less frequently limited by N or P compared with CR and was not nutrient-limited in 67% of all streams (Fig. 2). In reference streams, we found that GPP was never N-limited, and significant nutrient limitation of GPP by N or P was only found in the SW and NC sites. In contrast to what we saw for CR, agricultural land use increased rather than decreased the proportion of streams with nutrient limitation of GPP. Agricultural land use often resulted in reduced channel shading (Table 1), which likely alleviated light limitation, resulting in higher primary production and nutrient demand, increasing the likelihood of significant nutrient limitation of autotrophs. Further, we found that quantum yield (QY, GPP normalized to PAR levels used during assay incubations) on organic substrata increased with in situ light availability as both increasing PAR (data not shown; simple linear regression, $r^2 = 0.121$, $p = 0.02$) and decreasing percent channel shading (data not shown; simple linear regression, $r^2 = 0.20$, $p = 0.003$). Un-

Fig. 3. The nutrient response ratio across ecoregions for (*a*) community respiration (CR) on organic substrata, (*b*) gross primary production (GPP) on inorganic substrata, and (*c*) chlorophyll *a* on inorganic substrata calculated as the ratio of N + P treatments to control treatments, where a ratio of one indicates no nutrient limitation. Means ± standard error (SE) are reported. Bars with different letters represent significant differences among ecoregions as determined by analysis of covariance (ANCOVA) with percent modified as the covariate followed by Tukey's post-hoc comparisons test on log-transformed data ($p < 0.05$).



like agricultural land use, urbanization did not increase the frequency of nutrient limitation, and the proportion of urban streams with no nutrient limitation of GPP by N or P was approximately the same as reference streams.

Similar to organic substrata, GPP on inorganic substrata was not nutrient limited by N or P in 75% of all streams pooled across land-use types (Fig. 4). In reference streams, significant nutrient limitation was slightly more frequent than when pooled across land-use types, and most of the reference streams with nutrient limitation of GPP were located in ecoregions with open canopies in native vegetation (i.e., WY, KS, and SW). Human land use reduced the frequency of nutrient limitation of GPP. In agricultural streams, GPP was never P-limited or co-limited by N + P; whereas in urban streams, GPP was never N-limited. Across land-use types, QY significantly increased with PAR and decreased with percent channel shading (data not shown; simple linear regression: PAR, $r^2 = 0.197$, $p = 0.002$; percent channel shading, $r^2 = 0.204$, $p = 0.002$), indicating that light availability influenced GPP, confounding the potential influence of nutrient limitation on biofilm autotrophs.

Because inorganic substrata had significantly higher GPP and chl *a* compared with organic substrata (Fig. 1), we examined the NRR for GPP on inorganic substrata to determine the magnitude of nutrient limitation across ecoregions and land-use types. Across ecoregions, the NRR for GPP was highest in open canopy systems in KS, SW, and WY and lowest in PR (Fig. 3*b*; one-way ANCOVA, $p = 0.002$), but the effect of percent modified land use on NRR was not significant (one-way ANCOVA, $p > 0.05$). As light availability appeared to influence nutrient limitation status, we examined the influence of PAR on the NRR of GPP and found that the magnitude of nutrient limitation increased as light became more available (Table 2).

Nutrient limitation of chlorophyll *a*

When pooled across land-use types, chl *a* on organic substrata was not nutrient-limited in 65% of all streams, similar to results for GPP on organic substrata (Fig. 2). In reference streams, we found that N limitation was most frequent, there was no significant P limitation of chl *a* on organic substrata, and reference streams where chl *a* was nutrient-limited were again located in ecoregions with open-canopied streams. In both agricultural and urban land use, chl *a* was rarely nutrient-limited, with an equivalent frequency of N limitation, P limitation, and N + P co-limitation. However, in contrast to reference streams, nutrient-limited agricultural and urban streams did not correspond to ecoregions with open canopies. Across land-use types, we found that chl *a* on organic substrata significantly increased with PAR and decreased with percent channel shading (data not shown; simple linear regression: PAR, $r^2 = 0.47$, $p < 0.001$; percent channel shading, $r^2 = 0.33$, $p < 0.001$). Therefore, light availability was a strong driver of algal biomass, potentially obscuring the effects of land use on nutrient limitation.

On inorganic substrata, when data from all land uses were pooled, chl *a* was not nutrient-limited by N or P in 75% of all streams (Fig. 4). In reference streams, chl *a* was never P-limited and was only nutrient-limited in ecoregions with open canopy streams. In comparison with reference streams, human land use slightly relieved nutrient limitation of chl *a*, and both agricultural and urban land use showed similar patterns of nutrient limitation. Similar to results for chl *a* on organic substrata, land use likely has a mixed effect on nutrient limitation (high light and nutrients), making it difficult to identify causal factors for the patterns in nutrient limitation of chl *a* biomass. Across land-use types, chl *a* significantly increased with PAR and decreased with percent channel shading (data not show; simple linear regression: PAR, $r^2 = 0.31$, $p < 0.001$; percent channel shading, $r^2 = 0.297$, $p < 0.001$), indicating again that light availability had a strong effect on chl *a* biomass on inorganic substrata.

We also compared NRR for chl *a* on inorganic substrata across sites and found that like GPP, NRR was highest in

Table 2. Significant simple linear regressions with the nutrient response ratio (NRR) of community respiration (CR) on organic substrata, gross primary production (GPP) on inorganic substrata, and chlorophyll *a* (chl *a*) on inorganic substrata.

Dependent variable	Independent variable	r^2	*p* value	*n*
CR on organic substrata (log-transformed)	NH_4^+ concentration (log-transformed)	0.177 (–)	0.001	62
CR on organic substrata (log-transformed)	% modified land use (% agriculture + urban, arcsin $x^{1/5}$-transformed)	0.143 (–)	0.003	62
GPP on inorganic substrata (log-transformed)	PAR ($x^{1/3}$-transformed)	0.105 (+)	0.035	41
Chl *a* on inorganic substrata (log-transformed)	PAR ($x^{1/4}$-transformed)	0.240 (+)	<0.001	65

Note: All data were transformed as indicated in parenthesis. The type of relationship, either positive or negative, is indicated in parenthesis by the r^2.

Table 3. Mean nutrient response ratio of community respiration (CR) on organic substrata for reference, agriculture, and urban streams from each site (±1 standard error, SE).

Site	Effect of land use	Reference	Agriculture	Urban
KS	Strong	2.43 (0.88)	1.02 (0.10)	0.80 (0.05)
MA	Weak	0.96 (0.29)	1.46 (0.21)	1.70 (0.21)
MI	Moderate	1.64 (0.27)	1.28 (0.12)	1.51 (0.47)
NC	Strong	3.62 (0.47)	1.94 (0.07)	1.91 (0.20)
OR	Strong–moderate	2.29 (0.08)	1.27 (–)	1.61 (–)
PR	Weak	1.80 (0.25)	1.68 (0.06)	2.13 (0.78)
SW	Moderate–weak	1.55 (0.06)	1.11 (0.12)	3.62 (–)
WY	Moderate	3.24 (0.75)	2.71 (0.42)	2.65 (0.82)

Note: Sites are classified by the effect of land use: strong indicates a >45% decrease in the NRR with land use, moderate indicates a 10%–45% decrease, and weak indicates a <10% decrease.

WY, lowest in PR, and approximately the same across all other sites (Fig. 3*c*; one-way ANCOVA, $p = 0.006$), but the effect of percent modified land use on NRR was not significant (one-way ANCOVA, $p > 0.05$). Similar to GPP, chl *a* NRR significantly increased with PAR (Table 2), and variation in NRR also increased with PAR, requiring log transformation to increase homoscedasticity. Therefore, the response of chl *a* to added nutrients on inorganic substrata was greatest when light availability was high, but increased variation during high light availability probably indicates a mixed effect of anthropogenic land use.

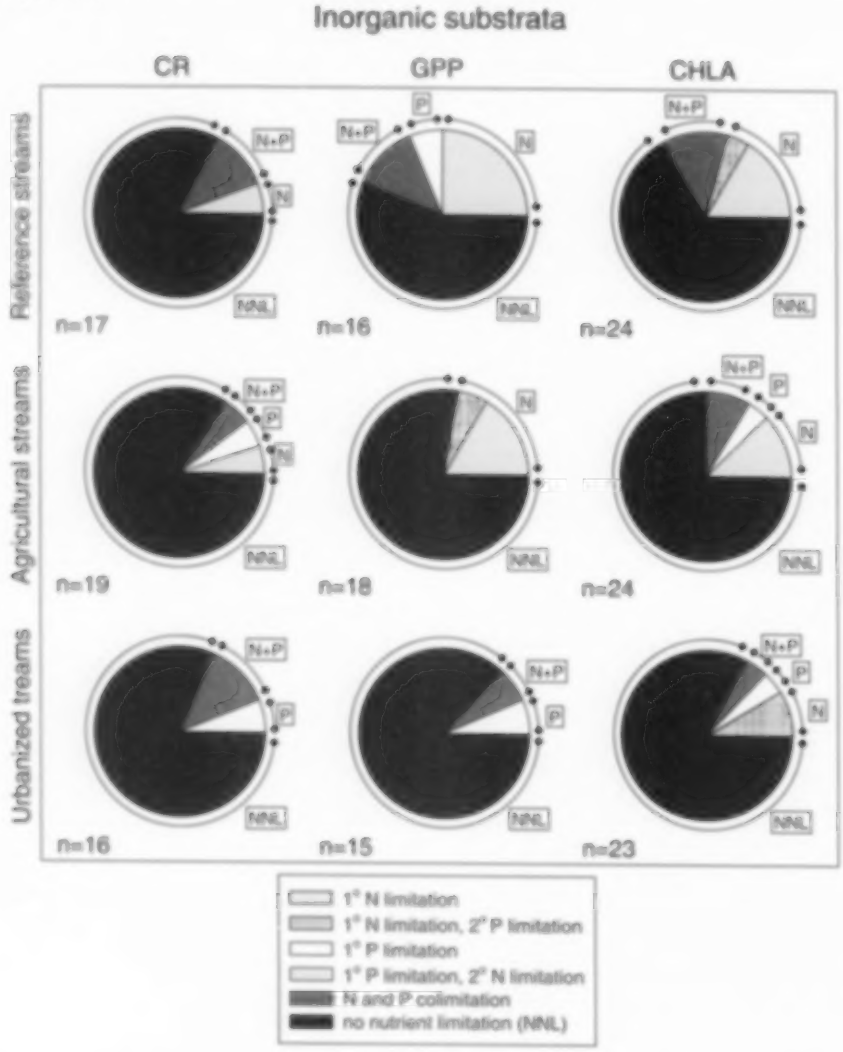
Discussion

Heterotrophic biofilms

Nutrient limitation of heterotrophic biofilms on organic substrata, as indicated by CR, was much more prevalent in reference streams than in agricultural and urban streams. We found significant nutrient limitation of heterotrophic biofilms in 23 out of 24 reference streams, which was a greater proportion than reported by Tank and Dodds (2003), who found that fungal biomass on wood veneers was nutrient-limited in six out of 10 relatively pristine streams across North America. There were two major differences between our results and those of Tank and Dodds (2003): (*i*) our process-related response metric (CR) was a more general heterotrophic response than their structural-based metric (ergosterol as an indicator of fungal biomass), and (*ii*) we used cellulose substrates, which were a more labile carbon source than their wood veneers. Several studies examining natural stream substrates have shown that leaf and wood breakdown (Meyer and Johnson 1983; Greenwood et al. 2007), leaf respiration (Stelzer et al. 2003), and fungal biomass (Grattan and Suberkropp 2001; Gulis et al. 2004) all increase with elevated nutrient concentrations in relatively pristine streams. Overall, these studies indicate that biomass and metabolism of heterotrophic biofilms are sensitive to water column nutrient concentrations and can potentially be influenced by changes in nutrient concentrations from natural or anthropogenic sources.

The frequency of heterotrophic biofilm nutrient limitation on organic substrata declined with agricultural and urban land use, yet the effect of land use was not consistent across all ecoregions. Although it is well documented that human land use influences stream ecosystems (Karr and Schlosser 1978; Paul and Meyer 2001), these effects often vary with land-use intensity (Allan 2004; Meyer et al. 2005). Across our ecoregions, there was high variation not only in the percentage of anthropogenic land use within stream basins, but also in the types of anthropogenic land use, with a net result of high variation in land-use intensity leading to variable stream nutrient concentrations. Typical background nutrient concentrations can change based on ecoregion because of differences in soils and climate (Omernik 1977; Smith et al. 2003), as exemplified by patterns in NO_3^- concentrations in our study, and these regional differences can affect the over-

Fig. 4. Nutrient limitation status of community respiration (CR), gross primary production (GPP), and chlorophyll *a* (CHLA) on inorganic substrata in reference (native vegetation), agricultural, and urbanized streams. Primary (1°) and secondary (2°) nutrient limitation status, as defined in the key, was determined by a significant response to nutrient treatment: N alone, P alone, and N and P added together (analysis of variance, ANOVA, $p < 0.05$).



all impact of agriculture and urban land use on streams. For example, we found that KS was highly impacted by human land use despite the low percent agricultural land use in each catchment, probably arising from a combination of typically low nutrient reference conditions and extensive fertilizer application associated with agricultural land use specific to that ecoregion. However, PR had high percent agriculture and urban land use in the impacted catchments, yet heterotrophic biofilms remained nutrient-limited by N and (or) P across all land-use types. Thus, across ecoregions, anthropogenic land use had a variable effect on streams, yet within ecoregions land use appeared to have a consistent effect, suggesting ecoregional differences in human land use (Omernik 1987).

On inorganic substrata, CR rarely showed significant nutrient limitation; heterotrophic colonization was likely suppressed by low carbon availability. Additionally, CR across all nutrient treatments was lower on inorganic substrata than on organic substrata, further suggesting carbon limitation. Heterotrophs on the inorganic substrata were likely dependent on an organic carbon source from the water column or from release by biofilm autotrophs (Olapade and Leff 2005). Autotrophic constituents of biofilms can support bacterial growth via algal exudates and cell death (Romani and Sabater 1999) and sometimes can be a source of inorganic nutrients (Geesey et al. 1978; Hepinstall and Fuller 1994). Additionally, previous studies suggest that heterotrophic biofilm activity is related to substrate carbon quality (Peterson et

al. 1993; Gessner and Chauvet 1994). Overall, heterotrophic nutrient limitation by N or P will be mediated by substrate carbon quality on which heterotrophic colonization occurs.

Measurement of CR includes both heterotrophic and autotrophic respiration. On organic substrata, CR appeared to be dominated by heterotrophic respiration because CR was much greater on organic substrata compared with inorganic substrata. In contrast, GPP showed the opposite pattern and was greater on inorganic substrata. Thus, large differences between substrata suggest that the high respiration on organic substrata is heterotrophic. The CR on inorganic substrata showed consistent results with GPP and chl *a*, suggesting that we captured a large fraction of autotrophic respiration in these particular measurements. We may have captured a response of autotrophs to nutrients via CR on inorganic substrata that was not significant with GPP or chl *a*.

Autotrophic biofilms

Light availability (as indicated by percent channel shade or PAR) appeared to be an important determining factor of the magnitude of autotrophic nutrient limitation across all land-use types and on both substrata. Autotrophic biofilms on inorganic substrata from reference streams were only nutrient-limited in ecoregions with naturally open canopies, and PAR and canopy cover were drivers of substratum GPP and chl *a* concentrations. Light often controls stream periphyton growth (Hill 1996; Mulholland et al. 2001; Bernhardt and Likens 2004) and nutrient response (Hepinstall and Fuller 1994; Larned and Santos 2000; Mosisch et al. 2001). Furthermore, human land use often influences both light and nutrient availability, obscuring the relationships between autotrophic biofilms and land-use changes (Karr and Schlosser 1978; Greenwood and Rosemond 2005; von Schiller et al. 2007). Our data support this possibility, as increasing percent modified land use led to increased NO_3^-, NH_4^+, and SRP concentrations and decreased percent channel shading, and we found an increase in chl *a* variability with high light availability.

However, a lack of autotrophic response to added nutrients could be associated with other factors besides light availability, especially the influence of invertebrate grazing and (or) substratum scouring as a result of high flows (Steinman 1996; Biggs and Close 1989). Direct observations of grazing by snails and amphipods on the organic substrata in particular occurred in a few streams, yet the proportion of streams that reported obvious herbivory was much less (9%) than those with no autotrophic nutrient limitation (65%). In most of the streams, nutrient diffusing substrata were deployed during base-flow conditions, resulting in a low potential for scouring of developing biofilms; nevertheless, the opportunity for small spates was possible. Finally, we may have been unable to detect a response of GPP to added nutrients if assay incubation PAR was significantly lower than ambient stream PAR. Yet, in 75% of the streams, neither chl *a* nor GPP on inorganic substrata showed a significant response to nutrients. Light limitation is the most parsimonious general explanation for these results.

The contribution of our data to the understanding of stream nutrient limitation

Our study provided substantially more data than previously published meta-analyses of chl *a* based NDS results, particularly by including human-dominated streams. Francoeur (2001) summarized 237 NDS experiments using chl *a* as a response metric and found that a majority (43% of the experiments) showed no N or P limitation. Tank and Dodds (2003) also included a literature survey in their discussion summarizing 172 NDS experiments with chl *a* and showed that N and P co-limitation was most prevalent (41% of the experiments). Our study showed that no limitation of chl *a* was most common (75% of all streams), which was a greater proportion compared with the two previous meta-analyses, probably reflecting the incorporation of anthropogenic land use and a wide range in light availability in our project design. Individuals researching periphytic algal nutrient limitation could be less likely to perform experiments in low-light streams and to publish negative results. Our study adds a new dimension to previous research focusing on autotrophic biofilm nutrient limitation by investigating a wider range of stream types via incorporation of anthropogenic impacts on the landscape.

Management implications

Our results indicated differences in the heterotrophic and autotrophic response to nutrient amendment, which has implications for the management of streams to prevent local and downstream eutrophication. Our data support Dodds' (2006) suggestion that autotrophs and heterotrophs should be assessed separately to determine overall trophic state (eutrophic or oligotrophic) of a stream. For example, a stream receiving nutrient inputs could be eutrophic based on heterotrophic indicators (high CR) but oligotrophic based on autotrophic indicators (low GPP) because of low light availability. The relative importance of the autotrophic and heterotrophic contribution to stream ecosystem function varies across ecoregions and land-use types and by season (which we did not examine here), so the predicted response of stream ecosystems to nutrient enrichment should include these differences when considering potential nutrient assimilation pathways.

Our data support previous research that found that light availability often was a primary control of autotrophs (Hill 1996; Mulholland et al. 2001; Bernhardt and Likens 2004), which can influence the autotrophic state of streams (Dodds 2006), particularly in terms of GPP (McTammany et al. 2007). We also suggest that carbon may be limiting for heterotrophs and thus changes in carbon supply (either dissolved or particulate) could have a large effect on heterotrophic nutrient status. Dodds (2006) proposed that even reduced riparian cover, which may decrease allochthonous organic matter inputs to streams, could have little effect on carbon availability in a stream because autotrophic activity would increase, thus adding new carbon (via carbon fixation) to the system. Similarly, Bunn et al. (1999) found that as GPP increased in response to reduced riparian cover associated with human land use in Australian streams, so did CR. Although their increase in CR could be due to algal respiration, it could also be from heterotrophs that were stimulated by increased algal carbon availability (Kaplan and Bott 1989). Thus, it is imperative to gauge the effect of land use on carbon availability, as well as nutrient availability (i.e., use a stoichiometric approach; Dodds 2007; Dodds and Cole 2007), to fully understand how heterotrophic nutrient status may be mitigated by other controlling factors.

Finally, our results support recent studies that suggest the importance of using ecosystem metrics to investigate stream health (Bunn et al. 1999; Fellows et al. 2006). Although our study does not measure reach-scale ecosystem processes such as whole-stream metabolism, our results imply that ecosystems processes, particularly CR on organic substrata, could be sensitive to the effect of land use. The NDS method has the benefit of separating substrate-specific functional responses to land use, complementing whole-stream metrics of function. Whole-stream GPP and CR can respond to increased agriculture (Bunn et al. 1999; Fellows et al. 2006); however, this response may be largely a result of changes in canopy cover as seen in other agricultural systems (McTammany et al. 2007). Meyer et al. (2005) found that although both NH_4^+ and P demand declined with increased urbanization in a catchment, GPP and CR were not related to indicators of urbanization. Therefore, the effect of human land use on stream ecosystem health may lead to a variety of responses, and future studies should combine metrics of nutrient uptake, whole-stream metabolism, and potential indicators of nutrient limitation such as NDS, thereby covering a range of response scales as an assessment tool.

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Potential methods for measuring the activity patterns and energy use of Atlantic cod (*Gadus morhua*)

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Abstract: As there are no commercially available acoustic telemetry devices for quantifying the swimming activity and activity-related metabolic expenditures of a wide range of marine species, we (*i*) examined the suitability of three methods (electromyography; sonomicrometry; and tail differential pressure tags (DPT)) for measuring the swimming speed of Atlantic cod (*Gadus morhua*), and indirectly, metabolic rate ($\dot{M}O_2$) and (*ii*) measured the activity pattern of free-swimming cod carrying the DPT. All three methods yielded significant relationships with swim speed during a critical swimming speed (U_{crit}) test. However, only the DPT was able to discern between swimming speed differences of 0.1 body lengths (BL)·s^{-1} and provide a strong relationship with $\dot{M}O_2$. Further, we found that free-swimming cod fitted with the DPT swam at an average speed of 0.33 BL·s^{-1}, the speed previously reported to result in minimal cost of transport for this species. While the DPT has considerable potential for assessing the bioenergetics of marine fishes, the swimming economy of tagged Atlantic cod was lower above 0.4 BL·s^{-1} as compared with untagged fish, and U_{crit} was reduced by 25%. These latter effects are likely related to the tag's present size (39 g) and design.

Résumé : Comme il n'existe pas dans le commerce d'appareil de télémétrie acoustique pour mesurer l'activité de nage et les dépenses énergétiques reliées à l'activité chez une gamme étendue d'espèces marines, nous avons (*i*) examiné la valeur de trois méthodes (l'électromyographie, la sonomicrométrie et les étiquettes de queue à pression différentielle (DPT)) pour mesurer la vitesse de nage de morues franches de l'Atlantique (*Gadus morhua*) et indirectement leur taux de métabolisme ($\dot{M}O_2$) et (*ii*) déterminé les patrons d'activité en nage libre de morues porteuses de DPT. Les trois méthodes fournissent toutes des relations significatives avec la vitesse de nage lors d'un test de vitesse de nage critique (U_{crit}). Cependant, seules les DPT permettent de distinguer entre des différences de vitesse de nage de 0,1 BL·s^{-1} (longueurs du corps par seconde) et fournissent une forte relation avec $\dot{M}O_2$. De plus, les morues en nage libre porteuses de DPT nagent à une vitesse moyenne de 0,33 BL·s^{-1}, la vitesse déterminée antérieurement comme présentant le coût minimal de transport chez cette espèce. Alors que les DPT présentent un potentiel intéressant pour mesurer les données bioénergétiques chez les poissons marins, l'économie de la nage de morues franches de l'Atlantique porteuses d'étiquettes est réduite à des vitesses supérieures à 0,4 BL·s^{-1} par comparaison à celle de poissons sans étiquette et leur U_{crit} diminue de 25 %. Ces effets s'expliquent vraisemblablement par la taille actuelle (39 g) et la configuration des étiquettes.

[Traduit par la Rédaction]

Introduction

Research has shown that the energetics of fish are affected by a number of biotic and abiotic variables, including temperature, oxygen, food availability, reproductive state, and activity (Jobling 1994). Consequently, from the perspectives of fisheries management and aquaculture, as well as those of the energetics of fish locomotion and physiological ecology, an understanding of the activity and metabolic expenditure of fishes under varied environmental conditions is critical. Lab-based studies have shown that there is a well-defined relationship between swimming speed and energy use in many teleost fish species (e.g., Brett 1964; Webber et al. 1998; Behrens et al. 2006), and that although it is not possible to directly measure oxygen consumption in free-swimming fish, swimming speed can be determined using

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a number of parameters (e.g., tailbeat frequency, Lowe et al. 1998; Steinhausen et al. 2005; tail differential or integrated pressure, Webber et al. 2001; Steinhausen et al. 2007; and electromyography (EMG), Briggs and Post 1997; Geist et al. 2003; Lembo et al. 2007).

In fresh water, numerous researchers have used commercially available, implantable, EMG transmitters (Lotek Engineering, Newmarket, Ontario, Canada) to measure the swimming speed, and indirectly the oxygen consumption, of free-ranging teleost fishes (see Cooke et al. (2004) for a review). However, similar data is lacking for marine fishes for several reasons. First, the radiotelemetry signals from Lotek's EMG transmitters are rapidly dissipated in seawater, making them generally unsuitable for research on marine fishes (see Lembo et al. 2007 for a recent exception in a lab environment). Second, EMG tags with acoustic transmission capabilities have not been commercially developed, although some workers have used their own designs with success (Dewar et al. 1999). Third, acoustic telemetry devices developed for measuring swimming speed in sharks (speed-sensing and tailbeat transmitters, Sundström and Gruber 1998; Lowe et al. 1998) are not commercially available and are too large for most teleost fish species. Finally, differential tail pressure tags (DPT) with acoustic transmission capability, based on the research of Webber et al. (2001), have only recently become available (from Vemco, a division of Amirix Systems, Inc., Halifax, Nova Scotia).

The Atlantic cod (*Gadus morhua*) is a marine species of ecological and economic importance whose potential as an aquaculture species is increasing as wild stocks decline. Further, although a great deal is known about Atlantic cod metabolism and swimming capacity and activity (Nelson et al. 1994; Claireaux et al. 1995; Sylvestre et al. 2007), only one study (Steinhausen et al. 2007) has used telemetry to examine the relationship between these two parameters. Thus, this species is a good candidate for in-depth studies on the potential use of physiological telemetry devices for estimating the activity and metabolism of free-swimming marine teleost fishes. In this study, we used DPT, with internally (intraperitoneally) implanted acoustic transmitters, to further evaluate the applicability of this technology for measuring the swimming speed and energetics of free-ranging cod and to record the pattern of swimming activity of free-ranging cod in a large tank. These studies were important, as Steinhausen et al. (2006) showed that placing the transmitter body of the DPT externally may increase swimming-related metabolism above 0.8 body lengths ($BL \cdot s^{-1}$) and decrease critical swimming speed (Steinhausen et al. 2007). Further, we used "hard-wired" fish to examine the potential of two other physiological parameters for measuring cod swimming speed, and indirectly, oxygen consumption: (*i*) EMG, which has proven successful for quantifying the swimming activity of a number of freshwater fish species (Cooke et al. 2004) and (*ii*) sonomicrometry, which measures changes in muscle length with each contraction. This latter technique has been extremely useful in understanding red muscle function and mechanical performance in a number of fish species, including cod (Coughlin et al. 1996; Hammond et al. 1998; Syme et al. 2008).

Materials and methods

This research conformed to the guidelines published by the Canadian Council on Animal Care (Olfert et al. 1993) and was approved by Memorial University's Institutional Animal Care Committee (Protocols 03-01-KG and 04-01-KG).

Experimental animals

All fish used in the study were obtained from a sea-cage facility at Northwest Cove (Hermitage Bay, Newfoundland) and transported to the Ocean Sciences Centre (Memorial University of Newfoundland, St. John's, Newfoundland). The Atlantic cod used for the EMG study (994.6 ± 95.5 g; range = 743–1314 g) were maintained in ~10 m^3 tanks supplied with aerated seawater at 10 ± 1 °C, and photoperiod was maintained at ambient levels. For the sonomicrometry study, 12 cod (621.3 ± 27.6 g; range = 501–797 g) were maintained in a ~3 m^3 tank supplied with recirculated (85%), aerated water at 10 ± 0.5 °C. Finally, the fish used for the DPT study (1723.3 ± 81.6 g; range = 1400–2680 g) were maintained in ~10 m^3 tanks supplied with aerated seawater at 10 ± 1 °C and an ambient (10 h light : 14 h dark) photoperiod. All fish were fed daily, to apparent satiation, with a commercial cod diet and held for at least 2 weeks before experiments began.

Surgical procedures

For each method of assessing swimming speed, fish were netted from their tank and placed in seawater containing tricaine methanesulfonate (MS-222, Finquel; 0.15 $g \cdot L^{-1}$) until ventilation ceased. The fish were then weighed and measured before being transferred to a surgery table, where oxygenated seawater containing MS-222 (0.055 $g \cdot L^{-1}$) was constantly pumped over their gills. All fish were transferred to a swim tunnel respirometer (see details below) following surgery and commenced ventilation almost immediately. Fish were then given at least 18 h to recover before the initiation of experiments.

Electromyography

The procedure for implanting the EMG electrodes was performed as described by Beddow and McKinley (1999). Briefly, bipolar EMG leads made from 0.005 inch (1 inch = 2.54 cm) coated stainless steel wire (California Fine Wire Company; Grover Beach, California) were inserted at 0.7 body lengths (BL) into the red muscle along the lateral line and also into the white muscle 2 cm above the lateral line, and a third EMG lead was inserted into the ventral body wall at 0.5 BL to measure background noise. All leads were sutured (with 1-0 silk thread, American Cyanamid Company, Pearl River, New York) and glued (Vetbond, 3M, Minnesota) in place. Once surgery was completed, fish were transferred to a custom built 81 L Blazka swim tunnel set at 0.3 $BL \cdot s^{-1}$ to recover.

Signals were amplified using a P511 pre-amplifier (Grass Instruments, Rhode Island) and recorded (sample rate 2 kHz) using a PC laptop running Labview software (National Instruments, Texas). Amplification was set at 20 000×, with the high and low filters set at 1 kHz and 3 Hz, respectively. A 60 Hz notch filter was used to remove mains interference. Data were collected for 2 min at each swimming speed, during which time the fish's swimming

behaviour was videotaped. Analysis determined the average, root mean square (RMS), and integrated (Bode) full-wave rectified voltage that was generated during free-swimming at each speed. The video recordings were used to select 1 min periods of free-swimming, where free-swimming was defined as the fish swimming away from the sides of the swim tunnel (i.e., avoiding boundary effects) and the back retention screen.

Sonomicrometry

Sonomicrometry measurements were taken from red muscle at two points along the length of the fish: 0.35 and 0.6 BL. In cod, the red muscle lies in a superficial layer beneath the lateral line. To implant the 2 mm piezoelectric crystals (Sonometrics Corporation, London, Ontario) for sonomicrometry, 18 g needles were used to puncture through the skin ~20 mm dorsal to the lateral line and then pushed ventrally just under the skin into the red muscle. The needle was then removed and the opening of the resultant hole was widened using iris scissors to allow the crystal to pass into the muscle. The crystal was then fed into the red muscle via the track left by the needle using fine forceps. Once the crystal was in place, the crystal lead was sutured to the body of the fish using 1–0 silk thread (American Cyanamid Company) as close to the puncture as possible. Using the same procedure, a second crystal was then implanted alongside the first, with pairs of crystals always 12 mm apart (±6 mm from either side of 0.35 or 0.6 BL), and crystal orientation was not necessary as the signal was omnidirectional. Once surgery had been completed, fish were transferred to a 142 L custom-designed swim tunnel with water speed set at 0.3 $BL{\cdot}s^{-1}$. Sonomicrometry leads were passed through a modified bung in the lid of the swim tunnel, which allowed it to remain gas tight during respirometry.

Sonomicrometry measurements were collected using a TRX Series 6 Digital Ultrasonic Measurement System connected to a PC running Sonoview (v.3.3.4) software (Sonometrics Corporation, London, Ontario, Canada). An analog representation of the sonomicrometry signals was in turn acquired by an MP100A-CE data acquisition system (BIOPAC Systems Inc., California), which was interfaced with a laptop PC running AcqKnowledge software (v 3.7.2; BIOPAC Systems Inc.) for data recording and later analysis. Data were collected for 1 min at each swimming speed at a sample rate of 2 kHz.

DPT

The V16-DP caudal DPT (Vemco Ltd., Shad Bay, Nova Scotia) consists of a pressure sensor body, a connecting lead (wire), the tag body, and attached battery (total mass 39 g). Several different methods for attaching the tag body and battery were tried in preliminary studies (external attachment (both dorsal and ventral) and intraperitoneal placement) to determine the method that had the least impact on the ability of the fish to swim. As a result of these experiments, it was decided to place the battery and tag body into the peritoneal cavity. Attachment of the caudal differential pressure sensor body was carried out as described by Webber et al. (2001). Briefly, a 18 g needle was passed through the caudal peduncle, and the sensor's positive port was passed through the aperture of the needle. The sensor body was then sutured (1–0 silk thread, American Cyanamid Company) in place, and the guide needle was removed. The lead connecting the sensor and the tag body was sutured loosely at ~0.75 BL on the left side of the fish. Then a 5 cm incision was made in the ventral surface of the fish (starting ~8 cm anterior to the anus), the sterilized tag body and battery were gently inserted into the body cavity, and the incision was sutured closed with 3–0 silk. Once surgery had been completed, fish were transferred to a 142 L custom-designed swim tunnel with water speed set at 0.3 $BL{\cdot}s^{-1}$.

Output from the tag was received using a hydrophone inserted into an aperture in the lid of the swim tunnel and connected to a VR60 receiver (Vemco Ltd.), which in turn downloaded data to a laptop PC running VSCAN software (Vemco Ltd.). This tag samples the pressure signal from the sensor placed on the tail at 30–40 Hz and converts it to an acoustic signal, which is relayed to the hydrophone. The time interval between pulses (420 ms minimum, 931 ms maximum) varies inversely with the pressure generated by the tail when swimming (i.e., the duration between pulses is 931 ms when the fish's tail is stationary) and is converted back into pressure using a simple equation by the software installed on the VR60. Data were collected for 10 min at each swimming speed, and a 5 min sample was used for subsequent data analysis.

Respirometry experiments on an additional group of fish, which were not subjected to surgery or tag implantation, were run in parallel with the fish fitted with the DPT. This additional group was swum to their U_{crit} (critical swimming speed; see calculation below) and used to determine whether tag attachment influenced swimming ability and (or) economy.

Finally, three fish outfitted with DPT were released into a 45 m^3 tank (7 m in diameter) with ≥50 conspecifics and given 24 h to recover from netting and handling. After this period of time, the VR60 receiver was used to collect data from the tags. Owing to the large amount of data created by the tags, 10 min periods were analyzed every 90 min, and these data were used to examine patterns in the fish's swimming speed and to estimate overall activity over a period of 8 h.

Respirometry

Swim trials were carried out the day after surgery by increasing current velocity from the baseline speed of 0.3 to 1 $BL{\cdot}s^{-1}$ in increments of either 0.1 $BL{\cdot}s^{-1}$ (for the sonomicrometry and DPT studies) or 0.125 $BL{\cdot}s^{-1}$ (for the EMG study). Each swim speed was maintained for 24 min, which included 5 min prior to respirometry to allow acclimation to the new speed, 14 min of respirometry during which time the inflow and outflow to the swim tunnel were closed, and 5 min after respirometry with the inflow and outflow open to allow the water to return to full air saturation. Oxygen concentration was measured in mg $O_2{\cdot}L^{-1}$ using a Cell Ox 325 oxygen probe that was housed in a D201 flow cell (WTW, Woburn, Massachusetts). The flow cell was supplied with water from the respirometer by a Masterflex L/S peristaltic pump (Cole Parmer, Anjou, Quebec), and the oxygen probe was connected to a salinity and temperature

compensating oxygen meter (Oxi 330i, WTW). Oxygen consumption was calculated using the following equation:

$$\dot{M}O_2 = \frac{\Delta O_2\ V}{MT}$$

where $\dot{M}O_2$ is the fish's oxygen consumption (mg O_2·kg^{-1}h^{-1}), ΔO_2 is the change in water oxygen content (mg·L^{-1}), V is volume (L) of the respirometer, M is mass of the fish (kg), and T is measurement period (h).

For cod fitted with the DPT, U_{crit} was calculated using the following equation:

$$U_{crit} = V_1 + [(T_1/T_2)V_2]$$

where V_1 is the last velocity where the fish swam for the entire 24 min, T_1 is time (min) swum at final speed prior to exhaustion, T_2 is time between velocity increases (24 min), and V_2 is velocity increment (0.1 BL·s^{-1}).

Statistical and data analyses

Statistical analyses were performed using SigmaStat (v 3.0.1, SPSS, Chicago, Illinois). Second-order regression analyses were used to describe the relationships between (*i*) swimming speed and EMG activity (volts) for white muscle and the reference electrode, muscle strain, and differential pressure and (*ii*) all indirect measures of swimming speed (EMG voltage, strain, and tail differential pressure) and metabolic rate. However, a third-order regression was used to relate swimming speed with red muscle EMG activity because the fit was substantially better.

One-way repeated measures analysis of variance (ANOVA) and Holm–Sidak post hoc tests were used to compare values of EMG activity, muscle strain, and tail pressure (differential and integrated) between swimming speeds. Pearson's correlation analysis was used to examine the relationships between the three measures of swimming activity (EMG, strain, and DPT) and $\dot{M}O_2$. A two-way repeated measures ANOVA and a Bonferroni post hoc test were used to compare differences in $\dot{M}O_2$ between the tagged and untagged fish at each swimming speed. Finally, *t* tests were used to compare both maximum $\dot{M}O_2$ and U_{crit} values between tagged and untagged fish.

Raw DPT data, obtained from free-swimming fish over a period of 1 h, were plotted individually for each fish on scatter graphs using SigmaPlot (v 8.0, SPSS, Chicago, Illinois) so that the swimming pattern of individual fish could be appreciated. A total of 1 h of integrated pressure data for each fish (composed of 10 min measurements performed every 90 min over 8 h) were then converted into swimming speed using the integrated pressure vs. swimming speed relationship derived in the swim tunnel and used to determine the activity pattern of the free-swimming cod (i.e., we used this data to divide activity into categories based on the percentage of time spent swimming at specific speeds).

Results

The relationships between the various measured parameters and swimming speed were quite variable, ranging from nearly linear for red muscle EMG RMS between 0.3 and 1.0 BL·s^{-1} to distinctly curvilinear for differential and integrated pressures and muscle strain (Figs. 1*a*–1*d*). Regression (second- or third-order) analysis of the data showed that all of the measured parameters had significant relationships with swimming speed. However, of the three methods examined, analysis of variance indicated that only integrated and differential pressure (i.e., the DPT) allowed for statistical discrimination among all swimming speeds (Figs. 1*c* and 1*d*).

Pearson's correlation analysis revealed that all parameters, with the exception of white muscle RMS, were significantly ($P < 0.05$) correlated with oxygen consumption (Figs. 2*a*–2*f*) and that the strongest correlation by far was found with the two pressure measurements.

While it was apparent from the above analysis that the DPT was the most accurate method for measuring the swimming speed and oxygen consumption of cod, it was also clear that the maximum swimming speed of fish fitted with the DPT was less than that measured using the two hard-wired methods (Fig. 1). This finding was confirmed when directly comparing the swimming speed – oxygen consumption relationship for DPT-fitted cod with that of fish from the same population that were not subject to tag implantation or surgery. For example, the U_{crit} of tagged fish (0.65 ± 0.03 BL·s^{-1}) was approximately 25% less than that of untagged fish (0.85 ± 0.03 BL·s^{-1}) (Fig. 3*c*). Further, although maximum oxygen consumption was not affected (tagged, 167.89 ± 2.14 mg O_2·kg^{-1}·h^{-1} vs. untagged, 172.46 ± 4.58 mg O_2·kg^{-1}·h^{-1}), it was apparent that carrying the DPT decreased the cod's swimming economy (Fig. 3*b*).

A scattergram of data from 1 h of integrated pressure measurements using the DPT showed that values were below 40 Pa (i.e., <0.5 BL·s^{-1}, see Fig. 1*c*) for the vast majority of the time, but that there were occasional short periods of burst swimming activity (Fig. 4). Further analysis of the DPT data (Figs. 4*a*–4*d*) revealed that while individual fish varied in the distribution of their swimming speed, their mean swimming speeds were nearly identical (range 0.33–0.35 BL·s^{-1}) and that they spent between 40% and 50% of their time swimming at 0.3 BL·s^{-1} (mean value 45.95%) and only a small percentage of their time motionless (mean value = 3.69%) or burst swimming (mean value = 1.34%) (Fig. 5). Burst swimming was defined as >0.6 BL·s^{-1}, as this was the average maximum swimming speed measured in the U_{crit} trials.

Discussion

The present research is the first to directly compare the accuracy of several potential methods for estimating the swimming speed and oxygen consumption of marine fishes. In addition to determining which method is most suitable for this purpose, we will highlight the shortcomings of all three and give suggestions as to how they could be improved or best applied. All methods were found to have a significant relationship with swimming speed; however, muscle strain amplitude recorded by sonomicrometry and muscle EMG activity were shown to have a poorer relationship as compared with the DPT, and only the DPT allowed for statistical discrimination between differences in swimming speeds as small as 0.1 BL·s^{-1}. Furthermore, this latter method had, by far, the strongest correlation with oxygen consumption over the range of swimming speeds examined. Given the degree

Fig. 1. The relationship between swimming speed and several potential parameters for estimating the activity and oxygen consumption as Atlantic cod (*Gadus morhua*) were swum to exhaustion. (*a*) Root mean square electromyography (RMS EMG) recorded from red muscle ($y = -0.07x^2 + 0.5x - 0.08$; $r^2 = 0.30$, $P = 0.002$) (grey circles), white muscle ($y = 0.002x^2 + 0.07x + 0.01$; $r^2 = 0.55$, $P < 0.001$) (open circles), and control electrodes (solid circles) ($n = 4$–5). (*b*) Strain in anterior ($y = 0.09x^2 - 0.08x + 0.04$; $r^2 = 0.28$, $P = 0.005$) (solid circles) and posterior ($y = -0.06x^2 + 0.1x + 0.02$; $r^2 = 0.25$, $P = 0.003$) (open circles) red muscles ($n = 7$–8). (*c*) Integrated ($y = 254x^2 - 71x + 17$; $r^2 = 0.86$, $P < 0.001$) and (*d*) differential pressure ($y = 3093x^2 - 1704x + 332$; $r^2 = 0.85$, $P < 0.001$) recorded from Atlantic cod fitted with a differential pressure tag (DPT) ($n = 5$–6). All values are means ± standard error of the mean (SEM). On all graphs, symbols without a common letter indicate a statistically significant difference ($P < 0.05$) between data points.

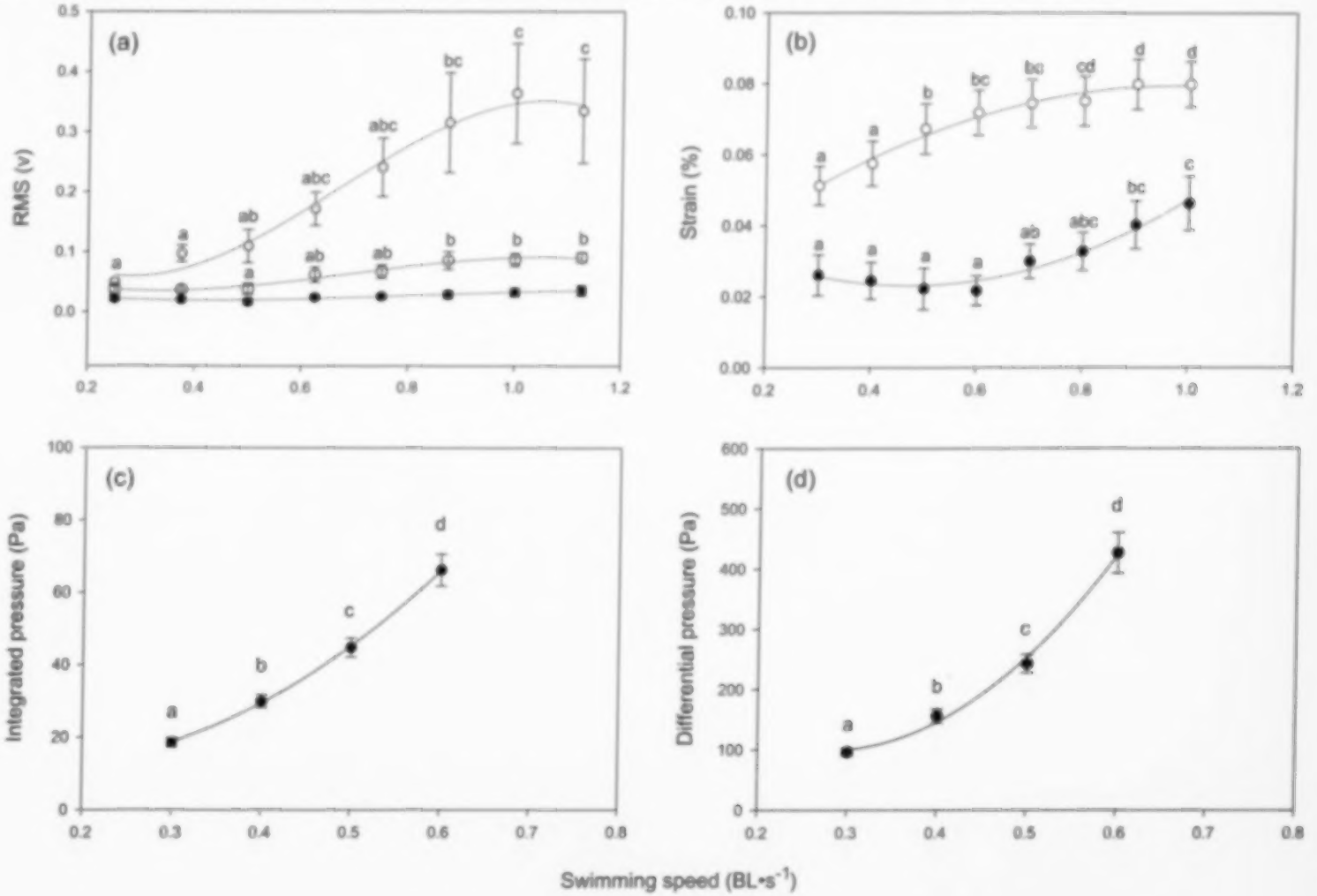

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Fig. 2. The relationship between oxygen consumption and red muscle (*a*) and white muscle (*b*) root mean square electromyography (RMS EMG) activity, anterior red muscle strain (*c*), posterior red muscle strain (*d*), integrated pressure (*e*), and differential pressure (*f*) as Atlantic cod (*Gadus morhua*) were exposed to increasing swimming speeds (increment 0.125 body lengths (BL)·s^{-1} or 0.1 BL·s^{-1}) until exhaustion. All plots were fitted with second-order regression lines and accompanying 95% confidence limits. Pearson's correlation coefficient (r) and P values are presented on each graph. See Fig. 1 for the number of fish utilized in each measurement.

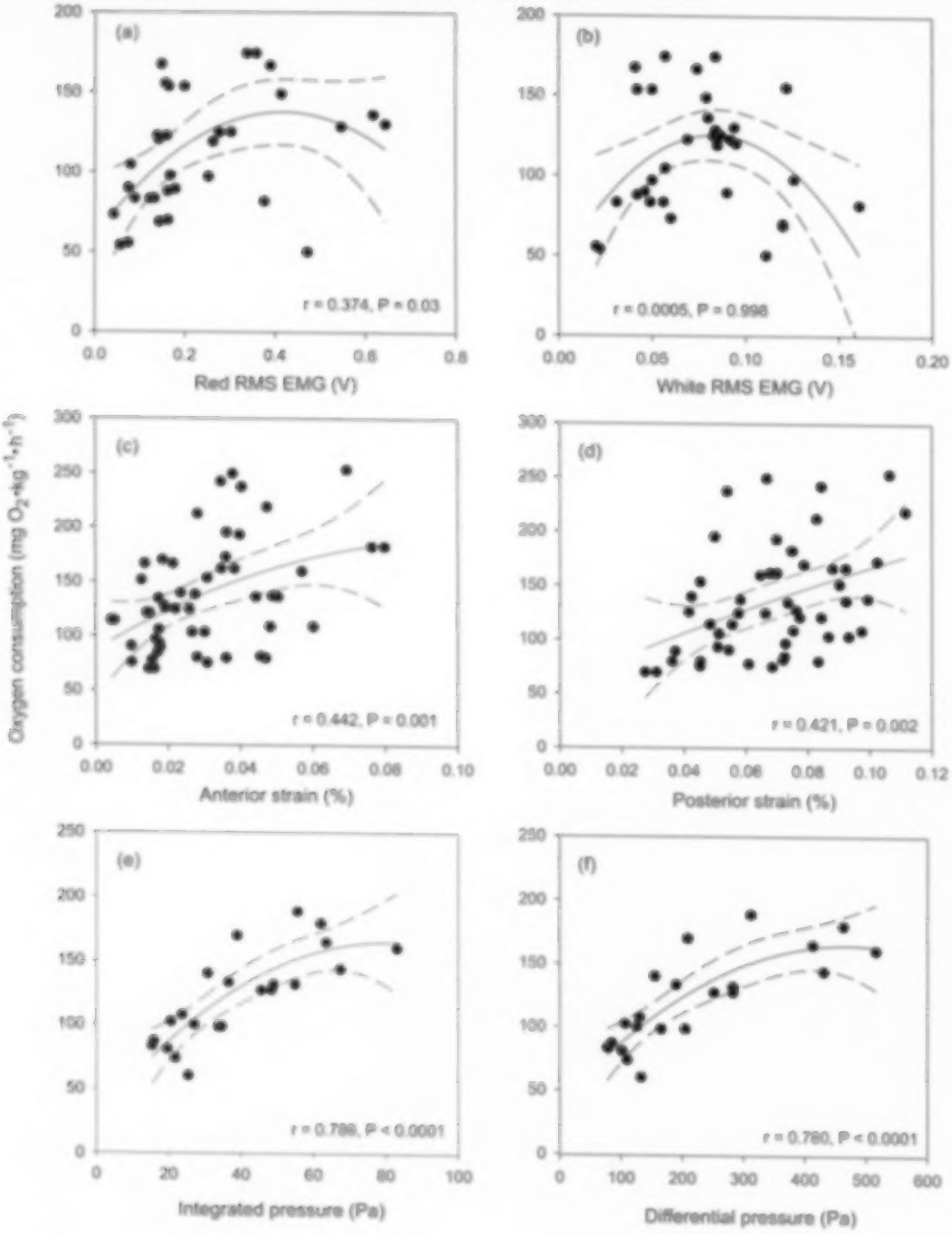
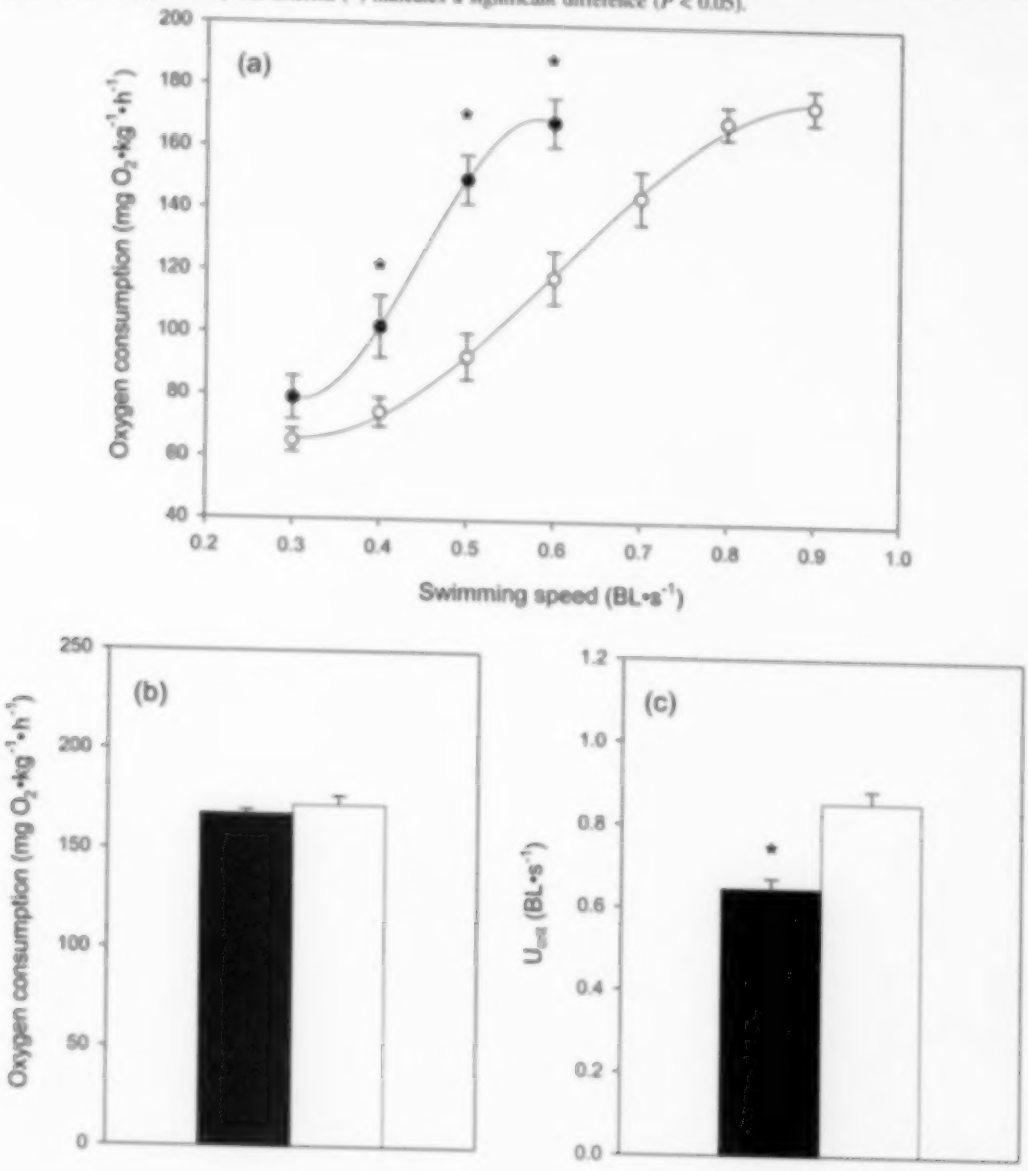


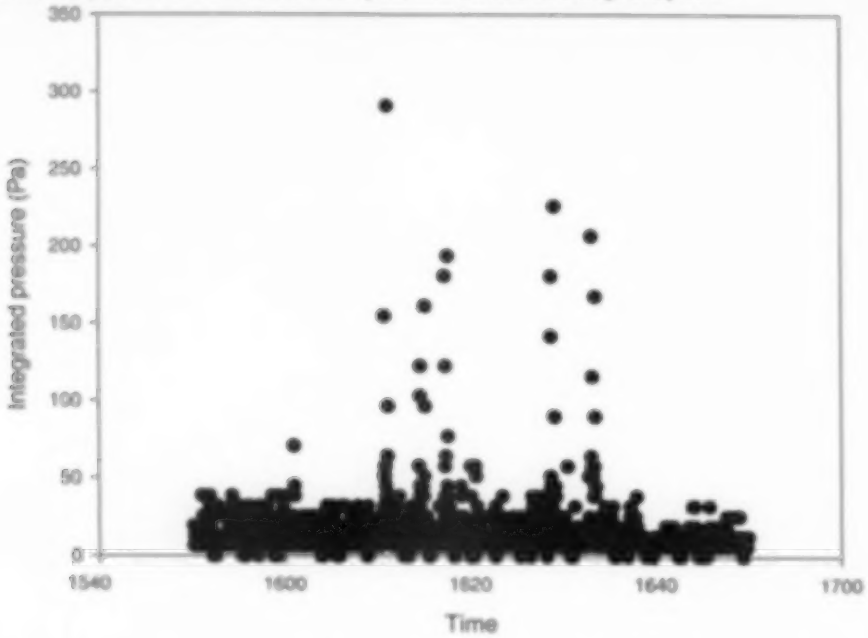
Fig. 3. (*a*) Comparison of oxygen consumption between Atlantic cod (*Gadus morhua*) fitted with the differential pressure tag (DPT) (solid circles: $y = -1031x^3 + 1873x^2 - 875x + 182$; $r^2 = 0.75$, $P < 0.001$) and untagged fish (open circles: $y = -9021x^3 + 12071x^2 - 4882x + 701$; $r^2 = 0.84$; $P < 0.001$). (*b*) Comparison of maximum oxygen consumption of tagged (solid bars) and untagged fish (open bars). (*c*) Comparison of critical swimming speed (U_{crit}) of tagged (solid bars) and untagged fish (open bars). All values are means ± standard error of the mean (SEM) ($n = 5$–8). An asterisk (*) indicates a significant difference ($P < 0.05$).



of accuracy provided by differential pressure sensors placed on the caudal peduncle, and the fact that all three studies that have used this device (Webber et al. 2001; Steinhausen et al. 2007; present study) report that differential pressure or integrated pressure are significantly correlated with swimming-related oxygen consumption, it appears that this method could be effectively utilized in future studies on the physiology and bioenergetics of free-swimming marine fishes.

Although the differential pressure sensor yielded the most reliable results for estimating the swimming speed and oxygen consumption of Atlantic cod, we found that the DPT (i.e., sensor + battery + tag) had a significant negative impact on the U_{crit} of Atlantic cod (25% reduction) and that

Fig. 4. Scattergram of integrated pressure recorded from an individual Atlantic cod (*Gadus morhua*) that was fitted with a differential pressure tag (DPT). The recording period was 1 h in total; a data point was recorded on average every 12 s.

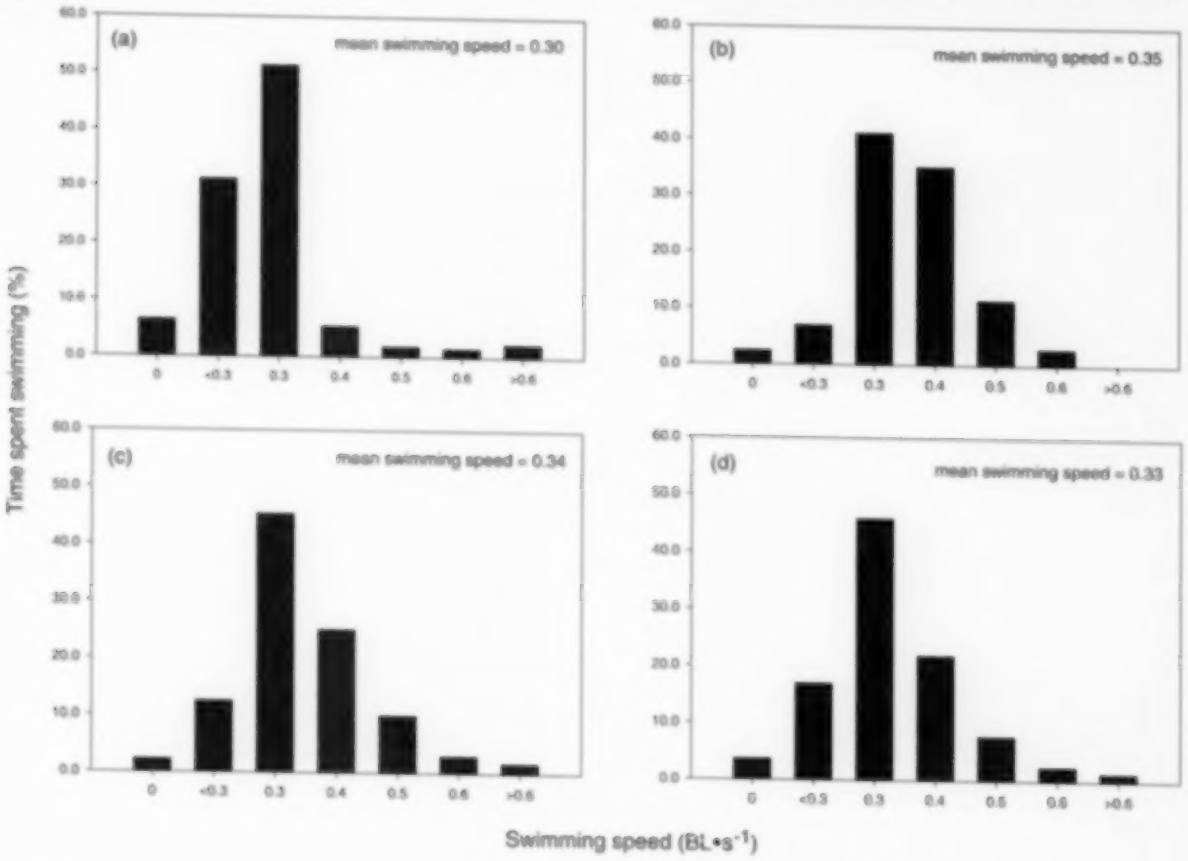


the $\dot{M}O_2$ of tagged fish was greater, as compared with untagged fish, at speeds above 0.3 BL·s^{-1} (i.e., swimming economy was reduced). Steinhausen et al. (2006) reported that U_{crit} decreased when a dummy tag of 37 g (~3% of body mass, BM) was externally attached to Atlantic cod. However, these authors only observed a significant effect of the tag on $\dot{M}O_2$ at velocities greater than 0.79 BL·s^{-1} and reported a decrease in U_{crit} of 13% (untagged = 1.14 BL·s^{-1}; tagged = 0.99 BL·s^{-1}). These authors suggested that the effect on U_{crit} and swimming-related $\dot{M}O_2$ was due to increased drag associated with external placement of the DPT body (tag + battery), and indeed, Lowe et al. (1998) found that externally placed tailbeat transmitters had a significant effect on swimming speed and oxygen consumption of juvenile scalloped hammerhead sharks (*Sphyrna lewini*). However, our data for cod with intraperitoneally placed tags indicates that increased drag cannot explain the negative effects of DPT attachment on swimming capacity and economy.

It has been suggested that the mass of telemetry tags should not exceed 2% of BM (Winter 1983; Jepsen et al. 2002); however, several experiments have used larger tags than this and shown no significant effect on the swimming performance of fish, including cod (Brown et al. 1999; Cote et al. 2004). While it is possible that the mass of the tags carried by the cod used by Steinhausen et al. (2006) (~3% of BM) resulted in the small reduction in U_{crit} reported, the large negative effects on U_{crit} and swimming economy reported here are not likely due to the mass of the DPT, as it was only slightly larger than 2% of mean BM. Instead, we propose that it was the combination of the large tag (given the limited space available in the cod's intraperitoneal cavity) and a limited time of postsurgical recovery (see fig. 7 in Webber et al. 1998) that resulted in reduced swimming economy and thus a lower U_{crit}. Further, this study is the only one of the three to use the DPT to derive relationships among swimming speed, oxygen consumption, and caudal pressure (Webber et al. (2001) and Steinhausen et al. (2006) used pressure sensors that were hard-wired to lab-based recording instruments), and thus, we cannot exclude the possibility that the wire connecting the tag to the pressure sensor on the caudal peduncle modified the swimming mode of the cod. This latter possibility would be consistent with McFarlane and McDonald (2002) and Peake and Farrell (2006), who suggest that the decreased swimming performance measured in U_{crit} tests is related to being forced to swim in an inefficient and unnatural manner at higher speeds (Peake and Farrell 2006). However, every effort was made to prevent the wire from affecting swimming (i.e., an adequate length was available, and the holding suture was tied in a loop such that the wire could move freely).

Numerous tagging studies have been carried out on freshwater species — mainly salmonids — using EMG transmitters (see Cooke et al. 2004 for a review). Many of the limitations of this method were discussed in that review, and they also apply to the development of an acoustic tag. The present study is the first to monitor EMG activity in swimming Atlantic cod and shows that the EMG signal of red muscle has a near linear relationship with swimming speed between 0.4 and 1.0 BL·s^{-1}. While this result and recent data for sea bass (*Dicentrarchus labrax*, Lembo et al. 2007) suggest that an acoustic EMG tag could be used in telemetry studies of swimming speed and patterns of activity in marine fish, there was only a weak correlation between red muscle EMG and $\dot{M}O_2$. It has been suggested that the placement of EMG electrodes can have a significant effect

Fig. 5. Percentage of time Atlantic cod (*Gadus morhua*) spent swimming at specific velocities in a large (7 m diameter) tank: panels *a*–*c* represent individual fish; panel *d* shows the mean values. The duration of the recording was 8 h, and data were collected for 10 min every 90 min.



on the output signal (Beddow and McKinley 1999); however, in the present study, the suggested optimal location of 0.7 BL for red muscle measurements was used. Thus, we believe that the poor relationship between EMG output and $\dot{M}O_2$ was likely due to (*i*) the level of noise that was associated with these measurements and (or) (*ii*) the lack of consistent function, and thus $\dot{M}O_2$, of red muscle during cruise swimming in cod (Syme et al. 2008). Although the change in white muscle EMG RMS was small (~0.2 V) as compared with the red muscle (~3 V), white muscle RMS became significantly elevated by 0.9 BL·s^{-1}. This indicates that white muscle makes a notable contribution to cod propulsion beginning at approximately 80% of U_{crit}, a value consistent with that reported for the rainbow trout (*Oncorhynchus mykiss*, Burgetz et al. 1998), but somewhat higher than observed for the sea bass (Lembo et al. 2007).

Dewar et al. (1999) described the development of an acoustic EMG tag and receiver for use on marine fish that was lab-tested on a range of species (e.g., Pacific bonito (*Sarda chiliensis*), yellowfin tuna (*Thunnus albacares*), spiny dogfish (*Squalus acanthias*), oyster toadfish (*Opsanus tau*)) and found that the EMG waveform could be transmitted and replicated with the accuracy required for complex quantitative analysis of muscle function. Our results for cod support their conclusion that red muscle EMG signals are accurate for quantifying muscle function and swimming activity. However, because of the lack of a strong relationship between EMG activity and $\dot{M}O_2$, it is unclear if further refinements to EMG technologies will permit the effective use of EMG tags for the quantification of marine fish bioenergetics.

Sonomicrometry has proven to be an effective method for examining changes in both the utilization and power output of different fish muscle types both in vivo and in vitro (Coughlin et al. 1996; Hammond et al. 1998; Syme et al. 2008). However, this technique has not, to our knowledge, been used to estimate fish swimming speed and (or) oxygen consumption in laboratory- or field-based experiments. Although there were significant relationships between both anterior and posterior muscle strain and cod swimming speed, and differences between these relationships at the two locations provide valuable insights into the functional role of anterior vs. posterior red muscles in this species (Syme et al. 2008), the association between muscle strain and oxygen consumption was highly variable, and this resulted in very weak correlations between the two parame-

ters. While it is possible that an optimum position for crystal implantation could be found (as suggested for EMG readings by Beddow and McKinley (1999)), which might afford more accurate estimates of cod (fish) metabolism during swimming, owing to the complexity, cost, and lack of a good fit, we feel that this technique has the least potential of the three examined for further development in the context of physiological telemetry.

Acoustic telemetry has been used to monitor the activity, behaviour, and physiology of a number of free-ranging fish species: e.g., rainbow trout (Briggs and Post 1997; Bégout Anras and Lagardère 2004), Atlantic cod (Steinhausen et al. 2007), northern pike (*Esox lucius*) (Armstrong et al. 1989), and scalloped hammerhead sharks (Lowe et al. 1998), to name a few. The monitoring of free-ranging marine species with acoustic tags can be a time-intensive exercise, as they generally have to be tracked with a hydrophone at relatively close range (Lowe et al. 1998; Nakano et al. 2003). As an alternative to acoustic tags, archival tags can be used; however, retrieval of these tags requires either fishing effort, which rarely yields high return rates, or the use of pop-up satellite tags, which are often prohibitively expensive (e.g., Marcinek et al. 2001; West and Stevens 2001; Stokesbury et al. 2007). It is possible with territorial species to use hydrophone arrays in the wild (Parsons et al. 2003; Clements et al. 2005; Topping et al. 2006), and analogous to this, cultured species offer an ideal opportunity to instrument and monitor fish behaviour and physiology. However, surprisingly little research has been carried out with regards to using acoustic tags to monitor cultured species (Baras and Lagardère 1995; Bégout Anras and Lagardère 2004; Steinhausen et al. 2007). In the present experiment, we monitored the swimming of three free-ranging cod in a large aquaculture holding tank for a period of 8 h using DPTs. It should be noted that this was a preliminary experiment and that stocking density was much lower as compared with typical broodstock tanks or sea cages (8–15 $kg \cdot m^{-3}$). Nonetheless, our results show that the majority (mean = 45.9%) of time was spent swimming at ~0.33 $BL \cdot s^{-1}$ and that only a very small proportion of time (mean = 1.3%) was spent burst swimming (i.e., at >0.6 $BL \cdot s^{-1}$). These data (Figs. 4 and 5) fit very well with previous data collected on this species. For example, Syme et al. (2008) report that the swimming velocity where the net cost of transport for adult cod is at a minimum is 0.3 $BL \cdot s^{-1}$. Further, although Björnsson (1993) reported that the mean swimming speed of cod varies considerably (0.2–0.55 $BL \cdot s^{-1}$) with the level of feeding, the distribution of swimming speed in cod fed submaximal rations was similar to that observed in this study. Thus, if our 8 h recordings were representative of daily swimming patterns, these data suggest that metabolic expenditures related to activity are kept to a minimum by free-swimming cod under land-based aquaculture conditions and that energy allocation during swimming could be estimated with reasonable accuracy using the DPT. This latter conclusion is based on the observations that (*i*) bursts of activity where integrated pressure was above the range measured in the swim tunnel experiments (~0–70 Pa) were very infrequent; (*ii*) swimming velocities above 0.4 $BL \cdot s^{-1}$ only accounted for 11.6% of total activity, and overestimates of oxygen consumption at these speeds could be corrected using data such as those presented here; and (*iii*) the mean swimming speed recorded for free-swimming cod fitted with DPTs is very similar to that recorded for untagged fish at comparable temperatures (between 0.29 and 0.38 $BL \cdot s^{-1}$, Petersen and Steffensen 2003; 0.32 $BL \cdot s^{-1}$, Schurmann and Steffensen 1994). It is likely that the occasional burst events were related to interactions with conspecifics (courtship or aggression) and (or) sharp changes in direction. However, video monitoring of tagged cod will be required to determine which, and to what extent, these behaviours contributed to the large, but short-lived, increases in sensor integrated pressure.

In summary, we have very limited knowledge of the bioenergetics of free-swimming fish, particularly species inhabiting or migrating into seawater. This is partially because acoustic tag technology has not developed as rapidly as that of some radio frequency tags (e.g., EMG, Lotek Engineering Inc.). In this study, we assessed the potential of three methods (EMG, sonomicrometry, tail differential–integrated pressure (i.e., a DPT)) for measuring the swimming speed of adult cod and indirectly swimming-related metabolism. All three methods yielded significant correlations with swimming speed; however, only DPT output (integrated or differential pressure) could distinguish swimming speeds differing by 0.1 $BL \cdot s^{-1}$ and appeared suitable for estimating the metabolic rate of free-swimming fishes. Our findings echo the sentiment of both Webber et al. (2001) and Steinhausen et al. (2007), that the DPT has potential as a reliable method for measuring activity and oxygen consumption in Atlantic cod (*G. morhua*) and other marine fishes. However, a reduction in tag and battery size is a high priority, and the use of a thinner wire between the tag and pressure sensor may be advisable, if the DPT is to become an effective tool for biotelemetry studies. Further, it is advisable that an increased postsurgery recovery period be allowed before investigators use this technology to investigate the relationship between DPT output and fish bioenergetics. Finally, DPT output from three free-swimming fish held in a large tank indicated that Atlantic cod spend a large proportion of their time (>65%) swimming at ≤0.3 $BL \cdot s^{-1}$, close to speeds that minimize the net cost of transport, and very little time burst swimming (i.e., at speeds >0.6 $BL \cdot s^{-1}$). In the near future, we hope to instrument cod in aquaculture sea-cages to monitor fish under commercial conditions and to determine how their activity is altered by stocking density and environmental conditions such as temperature, season, and current speed.

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Mixed-fishery or ecosystem conundrum? Multispecies considerations inform thinking on long-term management of North Sea demersal stocks

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Abstract: Signatories of the 2002 World Summit on Sustainable Development declaration committed to maintain or restore fish stocks to levels that can produce the maximum sustainable yield (MSY), a goal that has been challenged on a number of grounds. The European Commission has stated an objective to manage fisheries (independently) to achieve MSY by 2015, which has catalysed the Regional Advisory Councils' (RACs) thinking on MSY and how it relates to their goal of developing long-term management plans. This study uses an ecosystem model of the North Sea to investigate questions relating to MSY in the context of mixed demersal fisheries for cod, haddock, and whiting. Results suggest that it is not possible to simultaneously achieve yields corresponding to MSYs predicted from single-species assessments and that the contradictory response of whiting is central to the trade-offs in yield and value for mixed demersal fisheries. Incompatibility between mixed-fishery and ecosystem-scale considerations exemplifies the difficult conceptual and practical challenges faced when moving toward an ecosystem approach.

Résumé : Les signataires de la déclaration du Sommet mondial sur le développement durable de 2002 se sont engagés à maintenir ou restaurer les stocks de poissons aux densités qui permettent de produire le rendement maximal durable (« MSY »), un objectif qui a été critiqué pour plusieurs raisons. La Commission européenne a mis de l'avant un objectif pour gérer les pêches (indépendamment) de manière à atteindre le MSY pour 2015, ce qui a catalysé la réflexion des Conseils consultatifs régionaux (RAC) sur le MSY et ses conséquences sur leur but de réaliser des plans de gestion à long terme. Notre étude utilise un modèle écosystémique de la mer du Nord pour examiner les questions relatives au MSY dans le cas des pêches benthiques mixtes de morues, d'aiglefins et de merlans. Nos résultats indiquent qu'il n'est pas possible d'obtenir simultanément les rendements correspondant aux MSY prédits par les évaluations faites sur les espèces individuelles et que les réactions contradictoires du merlan sont au centre des compromis dans le rendement et la valeur des pêches commerciales mixtes de poissons benthiques. L'incompatibilité entre les considérations reliées aux pêches mixtes et celles obtenues à l'échelle des écosystèmes met en évidence les difficiles défis conceptuels et pratiques qui surgissent lorsqu'on adopte une approche écosystémique.

[Traduit par la Rédaction]

Introduction

The development of an ecosystem-based approach to fisheries management (EAFM) has been a long time in the making; driven by numerous international initiatives starting in 1982 with the UN Convention on the Law of the Sea (1982; www.un.org/Depts/los/index.htm) and reinforced most recently by the 2002 Johannesburg World Summit on Sustainable Development (WSSD; see Food and Agricultural Organisation (FAO) 2003). Other major international agreements that have led to the development of the EAFM include the Convention on Biological Diversity (1992; www.cbd.int/convention/), the Jakarta Mandate on Marine and Coastal Biological Diversity (1995; www.ngo.grida.no/wwfneap/Projects/Reports/jakmand.pdf), the Kyoto Declaration on the Sustainable Contribution of Fisheries to Food Security (1995; www.fao.org/DOCREP/006/AC442E/AC442e2.htm), and the FAO Code of Conduct for Responsible Fisheries (1995; www.fao.org/docrep/005/v9878e/v9878e00.HTM). In Europe, key political and legislative support for the EAFM comes from the Common Fisheries Policy (ec.europa.eu/fisheries/cfp_en.htm) and the Reykjavik Declaration on Responsible Fisheries in the Marine Ecosystem (ftp.fao.org/fi/DOCUMENT/reykjavik/y2198t00_dec.pdf). Other important European drivers for the EAFM are the European Union Action Plan for Biodiversity in Fisheries (europa.eu/legislation_summaries/environment/

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nature_and_biodiversity/128025_en.htm), the Bergen Declaration (www.ospar.org/html_documents/ospar/html/bergen_declaration_final.pdf), and the Oslo and Paris (OSPAR) Biodiversity Strategy(www.ospar.org/html_documents/ospar/html/Revised_OSPAR_Strategies_2003.pdf).

Signatories of the WSSD declaration subscribed to an international political commitment to maintain or restore stocks to levels that can produce the maximum sustainable yield (MSY), with the aim of achieving these goals for depleted stocks on an urgent basis, by not later than 2015 where possible. The principle and likelihood of achieving MSY simultaneously for multiple stocks has been challenged on the grounds that the concept of MSY is embedded within the dynamics of biological, ecological, economic, and social processes and therefore unlikely to be a robust objective (Larkin 1977; Mace 2001; Punt and Smith 2001). Nonetheless, the European Commission has stated an objective to manage fisheries (independently) to achieve MSY by 2015 (European Commission (EC) 2006) and to move to longer-term management systems that deliver this. These objectives are aligned with the aim of the Common Fisheries Policy "to ensure exploitation of living aquatic resources that provides sustainable economic, environmental and social conditions and gradual implementation of an ecosystem approach to fisheries management."

The European Commission's objectives have fuelled debate on MSY and helped catalyze the Regional Advisory Councils' (RACs) thinking on the development of long-term management plans (Pope et al. 2006). Although partly intertwined with and influenced by MSY objectives, long-term management plans go beyond thinking about MSY. They aim to incorporate broader goals of the RACs to achieve sustained, profitable fisheries that support fishing as a livelihood. (Note that RACs are stakeholder-led bodies (EC 2004) that submit recommendations and suggestions to the Commission on matters relating to fisheries management.)

The North Sea RAC (NSRAC) has focused its attention on the challenge of developing long-term management plans for the "mixed-demersal fishery" that targets cod, haddock, and whiting. For many years, the total allowable catch (TAC) management system has failed to deliver intended reductions in fishing mortality (F) in this fishery, the principal reason for this being that setting TACs independently for each stock only limits the amount of the catch that may be legally landed. It does not explicitly control the amount of catch and, hence, fishing mortality rate on each stock, because fishing continues and extensive discarding occurs as long as the TAC for at least one stock has not been reached. Discussion between the NSRAC and fisheries scientists has led to a series of logical questions. (1) What level of F is likely to result in MSY for North Sea demersal stocks and how does this equate to fishing effort? (2) Can MSY targets for individual species be achieved simultaneously for North Sea demersal stocks and within the specified time scale? (3) What is likely to happen to yield and revenue in each of the main fisheries in attempting to reach candidate management targets? (4) How might the North Sea stocks of cod, haddock, and whiting and the fisheries exploiting them be affected when environmental effects are included as a driver of the stock dynamics? The purpose of this work is to address the four questions above and in doing so to investigate the interrelated dynamics of cod, haddock, and whiting stocks and their response to fisheries in an ecosystem context.

Similarities in the ecology of cod, haddock, and whiting and their interaction with fishing gear means that it is very difficult to regulate independently the fishing mortality for each species. It has long been recognized that such considerations are critical in determining the yield dynamics of mixed-demersal fisheries and should be taken into account when formulating long-term plans and rebuilding strategies (International Council for Exploration of the Sea (ICES) 1997), but doing so is not easy. It requires knowledge of (*i*) differences in compensatory response, the degree to which reductions in stock size are compensated for by increases in juvenile survival rates (note: the ecological basis for sustainable net production) (Walters and Korman 1999; Walters and Martell 2004), (*ii*) predator–prey interactions, and (*iii*) fishery interactions. In this investigation, such information (see Appendix A) is incorporated into an ecosystem model of the North Sea (Mackinson and Daskalov 2007). The ecosystem model is calibrated to historical data and used to estimate F_{MSY} and MSY for cod, haddock, and whiting and to evaluate the likely consequences of implementing EU-defined management targets to achieve MSY for the mixed-demersal fisheries in the North Sea. Broader ecosystem-scale consequences, including the effects of environmental change, are also elaborated upon.

Materials and methods

Description and calibration of the North Sea ecosystem model

The model of the North Sea ecosystem (Mackinson and Daskalov 2007) is developed using Ecopath with Ecosim (EwE; Christensen and Walters 2004*a*; Christensen et al. 2004). It comprises 68 functional groups including mammals (3), birds (1), fish (45), invertebrates (13), microflora (2), phytoplankton (1), discards (1), and detritus (2). Commercially important fish species are divided into juvenile and adult groups (e.g., cod, whiting, haddock, saithe, herring), and numerous other fish groups are represented at the species level where data allow. The model represents the year 1991 and is parameterised with estimates of biomass, production, and consumption rates and diet composition for each functional group using data derived from various sources, the principal ones being ICES international bottom-trawl surveys for fish, international benthos surveys for epifauna and infauna (Craeymeersch et al. 1997; Callaway et al. 2002), the 1991 "Year of the Stomach" stomach-sampling project (Hislop et al. 1997), ICES single- and multi-species stock assessments, working group reports for seabirds, sharks, and marine mammals, specific published studies for phytoplankton, zooplankton, and microflora, and empirical models (e.g., Brey 2001, benthos production model). Information on fisheries landings (STCF 1991 database; Vinther and Thomsen 1992) and discards (CEFAS Trio discard database) are grouped into 12 fleet categories defined by the EU Data Collection Regulations. Economic data on vessel costs and fish prices for each fleet are taken from STCF 1991 and STECF databases. Mackinson and

Daskalov (2007) provide a comprehensive description of the data sources.

Accepting that any model is a gross simplification of reality, how well the model represents the North Sea ecosystem and the robustness of its predictions depend on the quality of the input data and any assumptions relating to the formulation and parameterization of the static model in Ecopath and the time-dynamic simulations in Ecosim. Every effort was made to ensure that the Ecopath model parameters were within the ranges available from data; main departures from input data occurred in the benthos groups for which abundance estimates can be extremely variable due to limited sampling. The data-quality concern of most relevance for this study is the reliability of diet information (see Discussion). Parameterisation of Ecosim simulations through calibration to time series data serves as a further test of the sensitivity of both Ecopath and Ecosim parameters. Of particular importance to this study is the specification of parameters that determine the relative differences in average recruits per spawner, as this determines the relative productivity of each group (see Appendix B). The numerous iterations and dynamic tests undertaken during basic parameterisation of the model (see Mackinson and Daskalov 2007) have revealed that it is generally stable and robust. Key weaknesses are associated with groups for which the sources of mortality are poorly known, but these have little bearing on the analyses performed here.

Calibration of the ecosystem model involved estimating vulnerability parameters using a nonlinear fitting procedure during which Ecosim predictions were fitted to outputs of multispecies virtual population analysis (MSVPA; Andersen and Ursin 1977; Magnusson 1995) and other data for the period 1991–2004. During fitting, particular attention was made to ensure that the relative degree of compensation in recruitment for cod, haddock, and whiting predicted by the model was consistent with data from single-species models and MSVPA (Appendix A, Fig. A1). A description of the calibration procedure and analysis-supporting parameters used in the simulations is provided (Appendix B). For most species, the biomass trends predicted by the calibrated ecosystem model provided a reasonably good approximation to the MSVPA-estimated historical biomass trajectories and yield (Appendix B, Fig. B1). There were some differences in the predictions. In general, predictions from the ecosystem model are smoother, because not having detailed age structure, they do not account so well for large interannual variations and also because the dynamics are "buffered" across many more trophic groups than is represented in MSVPA. Of course, calibrating one model to another risks the danger of inheriting problems. Calibrating to survey data would be preferred, but initial efforts to do so were abandoned because of extreme variations in the data arising from the patchiness of sampling. Work is ongoing to fit the model using a combination of data from stock assessments and surveys.

Simulations using Ecosim

Using the calibrated Ecosim model, both dynamic and equilibrium simulations were used to address the four questions (Table 1). Dynamic simulations were used to predict the rate of change in biomass of each functional group over time as a function of fishing rate, growth rate, predation rate, and other losses. Equilibrium simulations were used to estimate single-species F_{MSY} for each harvested species by running a long simulation (1000+ years) in which F of the harvested species was incremented or decremented slowly, while holding all other Fs constant at the Ecopath base values and taking the F that resulted in the maximum average catch (i.e., MSY). During equilibrium analysis, the biomass of all other groups (not the target group) was either held constant (species interactions "off") or allowed to vary (species interactions "on"). When biomasses of groups are held constant, food availability and predation impacts are constant; in effect, the ecosystem model mimics a single-species stationary assessment with the biomass of the harvested group responding to changes in F. When biomasses of all groups are allowed to vary, the indirect knock-on effects of predator–prey interactions (trophic interactions) cause the biomasses of other groups to change, but only in response to fishing-driven changes in the biomass of the harvested species. Equilibrium simulations with species interactions "on" set the partial derivatives of the differential equations that define Ecosim with regard to fishing mortality equal to zero to identify the biomass values that would result from the continued application (100+ years) of the different levels of fishing mortality on a particular species. This analysis is called a full compensation assessment sensu Walters et al. (2005). The equilibrium analysis can also be performed by varying effort for different fleets, which is translated into a partial F for each species captured by that fleet.

Approaches used to address the questions

Question 1. What level of F is likely to result in MSY for North Sea demersal stocks and how does this translate to effort?

To demonstrate differences in the responses that might be expected from models that account for species interactions (ecosystem models) and those that do not (single-species models), comparison was made of the ecosystem models' equilibrium predictions of single-species F_{MSY} and MSY for cod, haddock, and whiting when species interactions were turned off and turned on. So that results from the ecosystem model could be considered within a broader contextual background, comparisons were made with estimations of F in the model baseline year (1991) and with predictions of F_{MSY} (or other comparable reference values) taken from the European Commission's paper (EC 2006), single-species assessments, and MSVPA. Estimates of F_{MSY} from MSVPA were derived from data in Collie et al. (2003) by multiplying estimates of relative effort at MSY with partial fishing mortalities and then summing to obtain total F.

To equate the species-specific F_{MSY} with the fishing effort, F_{MSY} values were first translated to a multiplier of relative effort that all combined fleets exert on a species by calculating $F_{baseline}$ (the relative effort, equal to 1 in baseline year) and F_{MSY} (the relative effort at MSY) to give

$$\text{Relative effort at MSY} = (1 \cdot F_{MSY})/F_{baseline}$$

Secondly, the total relative effort was partitioned to each species–fleet pair by multiplying the total change in effort on a species by the proportion of a specific fleet's catch of

Table 1. Overview of simulations used in analyses.

Simulation type	Setup	Outputs compared	Question addressed	Tables and figures
Equilibrium	F of harvested species incremented and decremented over 1000+ years simulation.	Effects of including species interactions	1	Fig. 1
	Biomass of nonharvested group either held constant (species interactions "off") or allowed to vary (species interactions "on")	F_{MSY} (F that results in maximum average catch)		Table 2
		MSY (maximum average catch)		Table 2
		Relative fishing effort equating to F_{MSY}		Table 3
	F of harvested species incremented and decremented over 1000+ years simulation.	Effects of applying the different implementation approaches	2	Fig. 2
	F applied independently and simultaneously for cod, haddock, and whiting and applied to all harvested species in the model			
Dynamic	Vulnerability estimates taken from the calibrated simulations (1991–2004).	Equilibrium biomass and time for biomass dynamics to stabilize	2	Fig. 3
	Start year is 2004	Change in catches of each species		Fig. 4
	Primary production assumed fixed for duration	Impact on biomass of other groups in the ecosystem		Fig. 5
	Duration of simulation is 100 years			
	Fishing mortality on each species is applied as constant harvest rate pattern based on F_{MSY} (or proxy) targets or F_{MSY} predicted by the model			
	As above, except	Change in biomass, catch, and value of species under various effort regimes	3	Figs. 6, 7
	Relative fishing effort for each fleet is applied as constant effort pattern and incremented in steps from 0 to 3	Catches and revenues of principal fleets		Fig. 8
	Primary production forcing is turned off in 1991–2004 simulation and model is refitted with new vulnerability estimates	With and without primary production forcing	4	Figs. 9, 10

Table 2. Comparison of ecosystem model predictions and fishing mortality target reference points from alternative sources.

	Ecosystem model		EC working paper			Single-species assessment reference point (2006)			MSVPA	
	$F_{baseline}$ (1991)	F_{MSY} (with species interactions on)	F (2004)	$F_{0.1}$	EC F_{max} (F_{MSY} proxy)	F (1991)	F_{lim}	F_{pa}	F (1991)	F_{MSY} (derived[a])
Cod (adult)	0.772	0.494	0.910	0.132	0.201	0.886	0.86	0.65	0.815	1.192
Haddock (adult)	0.915	0.668	0.320	0.202	0.321	0.944	1.00	0.70	0.799	1.317
Whiting (adult)	0.515	0.561	0.278[b]	0.268	0.300[c]	0.738	0.90	0.65	0.617	0.795

Note: F_{MSY} is the level of fishing mortality that will, on average, result in a stock size that produces the maximum sustainable yield. F_{max} is the fishing mortality that would produce the maximum yield from one recruit and is equal to F_{MSY} if recruitment is independent of the size of the parent stock. F_{MSY} is considered to be an upper limit for sustainable fisheries, and it is therefore not appropriate to use F_{max} as a proxy for F_{MSY} when there is a risk that F_{max} may be larger than F_{MSY}. For this reason and because estimates of F_{max} may be very uncertain, $F_{0.1}$ is widely used as a proxy for F_{MSY}. The technical definition of $F_{0.1}$ is the fishing mortality at which the expected yield increase from adding another unit of effort is one-tenth of the yield produced when the first unit of effort was launched on the formerly unfished stock. It is considered to be a more robust reference point if one is to manage toward F_{MSY} because there is a better chance that it is at or below F_{MSY} and the estimation is less influenced by uncertainties than the estimation of F_{max} (EC 2006, Annex B, technical background to MSY proxies). For details of MSY-related reference points, see Cochrane (2002). F_{lim} is the level of fishing mortality at which there is an unacceptably high risk that stocks will collapse; F_{pa} is a lower level of fishing mortality that offers a high probability that (F_{lim}) will never be reached.

[a]Derived from data in Collie et al. (2003) under the guidance of Morten Vinther.

[b]Not included in the EC paper, but based on 2005 assessment (ICES 2006).

[c]Value assumed to be between $F_{0.1}$ and F_{max} given in the report.

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cod, haddock, whiting in the baseline year of the model. A brief comparison is made between this simple translation of F_{MSY} to relative effort, with an equilibrium simulation where fishing effort is varied for demersal trawlers.

Question 2. Can MSY targets for individual species be achieved simultaneously for North Sea demersal stocks? And within the specified time scale?

Equilibrium simulations were used to make predictions of MSY when single-species F_{MSY} targets for cod, haddock, and whiting are applied simultaneously (mixed-fishery approach) compared with those when targets were applied independently (single-species approach). An additional test was made in which the predictions of MSY for cod, haddock, and whiting were evaluated when single-species F_{MSY} targets for all harvested species in the system were applied simultaneously (ecosystem approach).

To get an appraisal of the differences in response time between cod, haddock, and whiting and consider wider ecosystem impacts, dynamic simulations were used to predict the long-term (100 year) "equilibrium" response to the EC's proposed management targets (F_{MSY} proxies) and model-predicted F_{MSY} values. Time of recovery to a stable biomass was recorded for cod, haddock, and whiting. The impact on the biomass of other components of the ecosystem was examined.

Question 3. What happens to yield and revenue in each of the main fisheries, assuming candidate management targets?

In lieu of specific management targets proposed by the RACs, the responses of cod, haddock, and whiting to various levels of effort from the principal demersal fisheries (demersal trawl and seine and pelagic trawl and seine) were investigated using dynamic simulations. Consequences for the principal fleets and for the ecosystem as a whole were assessed in terms of changes in biomass, catch, and revenue of each species. These are captured through a series of three-dimensional effort response surfaces that depict responses to a combination of effort regimes. Economic factors are considered in Ecopath–Ecosim through inclusion of market prices for the harvested species and differential costs of fishing for each fleet, thus allowing comparison of revenue flows when evaluating alternative management strategies.

Question 4. What happens to the differential responses of cod, haddock, and whiting when environmental effects are included as a driver of the stock dynamics?

Recognising that environmental effects can play a key role in stock dynamics of gadoids (e.g., Drinkwater 2005), a hindcast simulation (1991–2004) was performed to demonstrate the possible effects that accounting for environmental influences on stock dynamics might have on the differential responses of cod, haddock, and whiting. These were implemented in the model by forcing changes in primary production based on historical data from the continuous plankton recorder (see Mackinson and Daskalov 2007, p. 71). A simple comparison is made of the time-integrated (monthly) biomass predictions from a parameterisation that does not include primary production forcing.

Fig. 1. Ecosystem model predictions of the equilibrium relationship between fishing mortality rate and yield of (*a*) cod, (*b*) haddock, and (*c*) whiting when the biomass of groups other than the harvested group is held constant (broken line, no species interactions, i.e., stationary assessment) and when species interactions are allowed to vary in response to changes caused by fishing on the target species (solid line, full compensation assessment). t, tonnes.

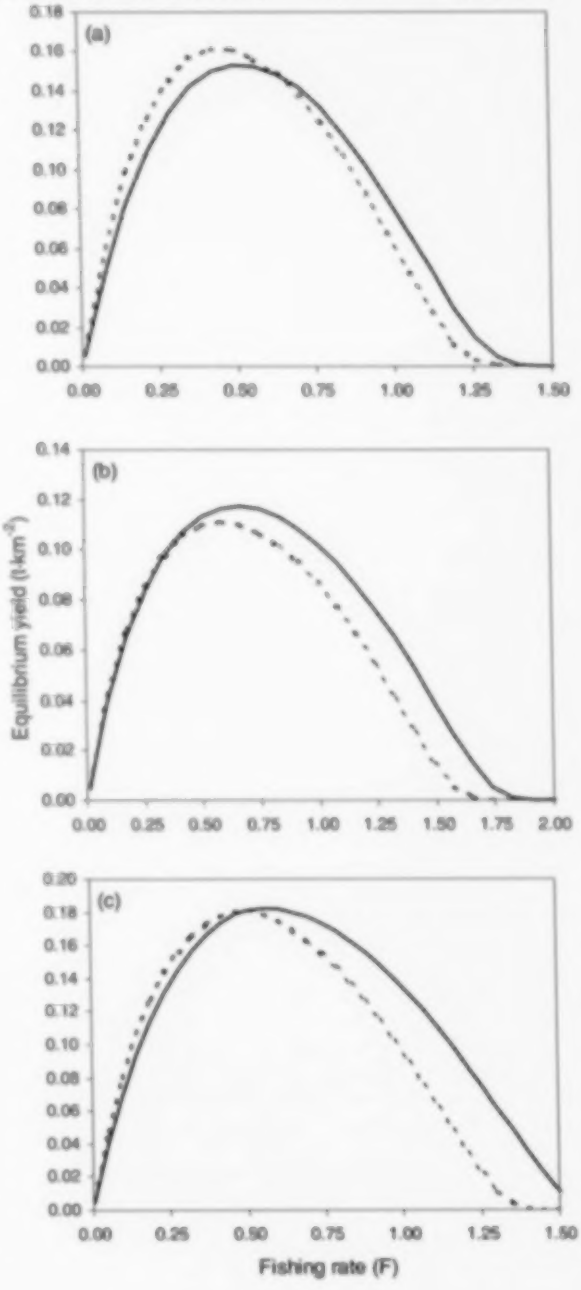


Table 3. Changes in relative fishing effort required to achieve the model predicted F_{MSY} (see Table 2).

	All gears combined	Demersal trawl and demersal seine	Pelagic trawl	Beam trawl	Sandeel trawl	Drift and fixed nets	Nephrops trawl	Other
Relative effort in the baseline year (equivalent to the proportion of each species caught by each gear)								
Cod (adult)	0.320	0.082	0.022	0.019	0.001	0.155	0.042	0.000
Haddock (adult)	0.178	0.047	0.029	0.000	0.004	0.004	0.081	0.012
Whiting (adult)	0.283	0.080	0.039	0.009	0.027	0.002	0.122	0.004
Total	0.78	0.21	0.09	0.03	0.03	0.16	0.25	0.02
Relative effort associated with F_{MSY}								
Cod (adult)	0.205	0.05	0.01	0.01	0.00	0.10	0.03	0.00
Haddock (adult)	0.130	0.03	0.02	0.00	0.00	0.00	0.06	0.01
Whiting (adult)	0.308	0.09	0.04	0.01	0.03	0.00	0.13	0.00
Total	0.64	0.17	0.08	0.02	0.03	0.10	0.22	0.01
Change in effort (relative to baseline)		0.83	0.87	0.79	1.03	0.65	0.89	0.82

Note: Principal gears for mixed-demersal fisheries are shaded.

Results

Question 1. What level of F is likely to result in MSY for North Sea demersal stocks and how does this translate to effort?

When species interactions are taken into consideration, predictions of F_{MSY} for cod, haddock, and whiting are slightly higher than when species interactions are not included, but predicted MSYs remain similar (Fig. 1). The finding that F_{MSY} tends to be higher when species interactions are accounted for is the same as the general findings of Walters et al. (2005), but does not confirm the assertion that productivity (maximum yield) of the harvested species always increases. In addition to the predator–prey interactions among cod, haddock, and whiting and the indirect food-web effects with other predators and prey, cannibalism (Appendix A, Table A3) has an important effect on the compensatory response of whiting and cod. At higher fishing rates, declines in biomass of adult cod and whiting due to fishing result in higher juvenile survival rates through reductions in cannibalism. At lower fishing rates, increases in adult biomass result in increased cannibalism. The net effect of including species interactions (allowing for compensatory responses) is that the rate of change in biomass for each species is slower, biomass is depleted more slowly at higher fishing, and thus higher levels are fishing can be sustained.

The ecosystem model predicts that to achieve MSY for cod and haddock, current fishing mortality ("current" being F in the base year 1991) should be reduced by roughly 40% and 30%, respectively. F_{MSY} for whiting is predicted to be slightly higher than F in 1991 (Table 2). These predicted F_{MSY} values are below the proposed precautionary fishing mortality rates (F_{pa}) based on single-species assessments, a comparison that is justified on the grounds that fishing mortality estimates for 1991 are similar from both models. The European Commission's proposed proxies for F_{MSY} are around half that predicted by the ecosystem model. In contrast, estimates of F_{MSY} from MSVPA are about double those predicted by the ecosystem model for cod and haddock, but similar for whiting. M. Vinther (DTU Aqua, Charlottenlund Slot, DK-2920 Charlottenlund, Denmark, personal communication, 2007) cautions that the derived MSVPA estimates are sensitive to the specification of stock–recruitment parameters. The same caution actually holds true for all the model estimates, because the stock–recruitment relationship defines the strength of compensatory increases in juvenile survival rate as adult stocks decline.

Relative fishing effort required to achieve F_{MSY} for each species is about 20% less for the principal fleets, demersal and pelagic trawl (bottom line of Table 3). However, total effort of sandeel trawlers increases. This is because whiting constitute the largest fraction of demersal species that are caught by this gear and an increase in F is required to achieve MSY for whiting. Total changes in the relative effort on each species equate proportionally to the total F_{MSY} predicted for each species, with effort being partitioned among gears according to the proportion of species caught in the baseline year. Note that this simple translation of changes in cod, haddock, and whiting fishing mortality to effort is not the same as performing an analysis of how equilibrium yield changes for different levels of effort for a particular gear. That analysis takes account of the complex interactions among all the species affected directly and indirectly by fishing of that particular gear. The prediction for demersal trawlers shows that maximum yield of cod to demersal trawlers (assuming effort of other gears remains the same) is achieved with about half the effort, and that at lower fishing rates on cod, haddock biomass increases over the long term, but whiting declines (mainly due to predation by cod). More detailed analyses of the trade-offs in effort are discussed later.

Question 2. Can MSY targets for individual species be achieved simultaneously for North Sea demersal stocks and within the time scale?

Results indicate that it is not possible to achieve yields equivalent to the "single species" MSYs predicted by the model when target F_{MSY}s of individual species are applied simultaneously. When F_{MSY} targets for cod, haddock, and whiting are implemented together (a mixed-fishery approach), predicted MSY of cod is higher than its corresponding individual-species MSY, but those of whiting and haddock are lower. Also, when F_{MSY} targets are implemented simultaneously for all harvested species (ecosystem-scale target), the predicted MSYs are different again; this time being much lower for cod, haddock, and whiting

Fig. 2. Predictions of maximum sustainable yield (MSY) when F_{MSY} predicted by single species are applied in a single-species (open bars), mixed-fishery (stippled bars), and ecosystem (solid bars) context.

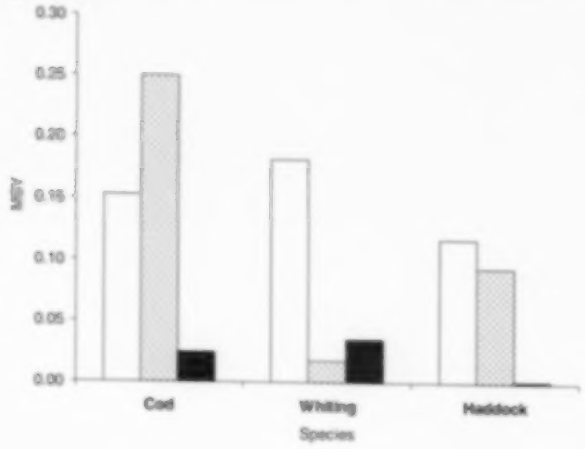
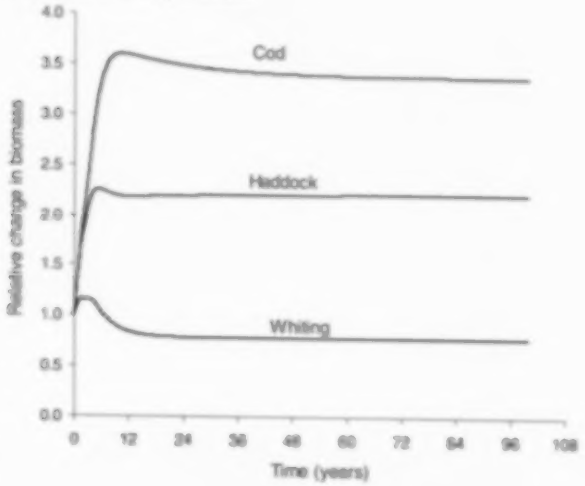


Fig. 3. Biomass trajectories when EC F_{MSY} proxy rates are applied simultaneously to cod ($F = 0.201$), haddock ($F = 0.321$), and whiting ($F = 0.3$) for a sufficiently long period for biomasses and catches to reach equilibrium.



(Fig. 2). These results indicate that the responses of cod, haddock, and whiting are influenced more broadly through their interactions with other species in the ecosystem and by the management actions for other fisheries. They bring into question the notion that the mixed fisheries for cod, haddock, and whiting can somehow be divorced from the dynamics of other system components.

In response to sustained fishing at EC-defined F_{MSY} proxies, applied simultaneously for cod, haddock, and whiting, equilibrium biomass of cod and haddock are 3.5 and 2 times higher, respectively, than in the base year (Fig. 3) and give rise to higher catches (Fig. 4). Whiting biomass declines as a consequence of increased predation by cod. Haddock were

Fig. 4. Changes in catch when EC F_{MSY} proxy rates are applied simultaneously to cod ($F = 0.201$), haddock ($F = 0.321$), and whiting ($F = 0.3$) for a sufficiently long period for biomasses and catches to reach equilibrium.

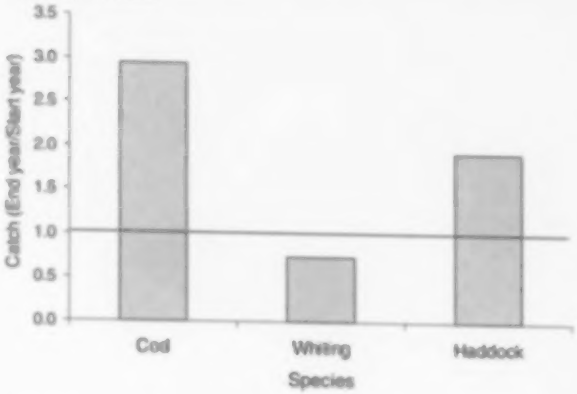
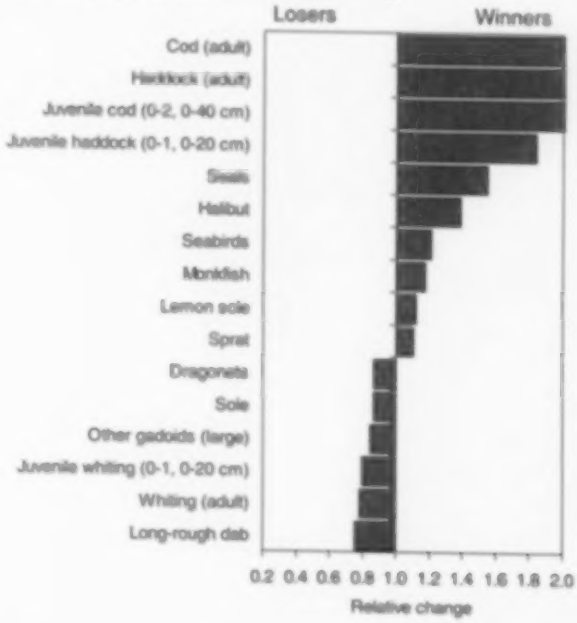


Fig. 5. Relative change in biomass (end/start) of selected (change > ±10%) functional groups in the ecosystem when EC F_{MSY} proxy rates are applied to cod, haddock, and whiting.



quickest to reach a new equilibrium biomass (11 years), whiting took twice as long (22 years), and cod took even longer (25 years) (Fig. 3). Differences in the relative response times demonstrate the importance of setting appropriate time frames for monitoring the effects of management actions. It is unlikely that all species will reach specified targets simultaneously. Such knowledge could be used for investigating how to design harvest strategies that are sympathetic (and adaptive) to differences in species biomass dynamics. Long-term changes in the biomass of other groups show how ecosystem structure is influenced (Fig. 5).

Fig. 6. Response curves showing long-term predictions (value in year 100) of equilibrium simulation biomass, catch, and value for (*a*–*c*) cod, (*d*–*f*) whiting, and (*g*–*i*) haddock. Note that Figs. 6*g*–6*i* appear on the next page.

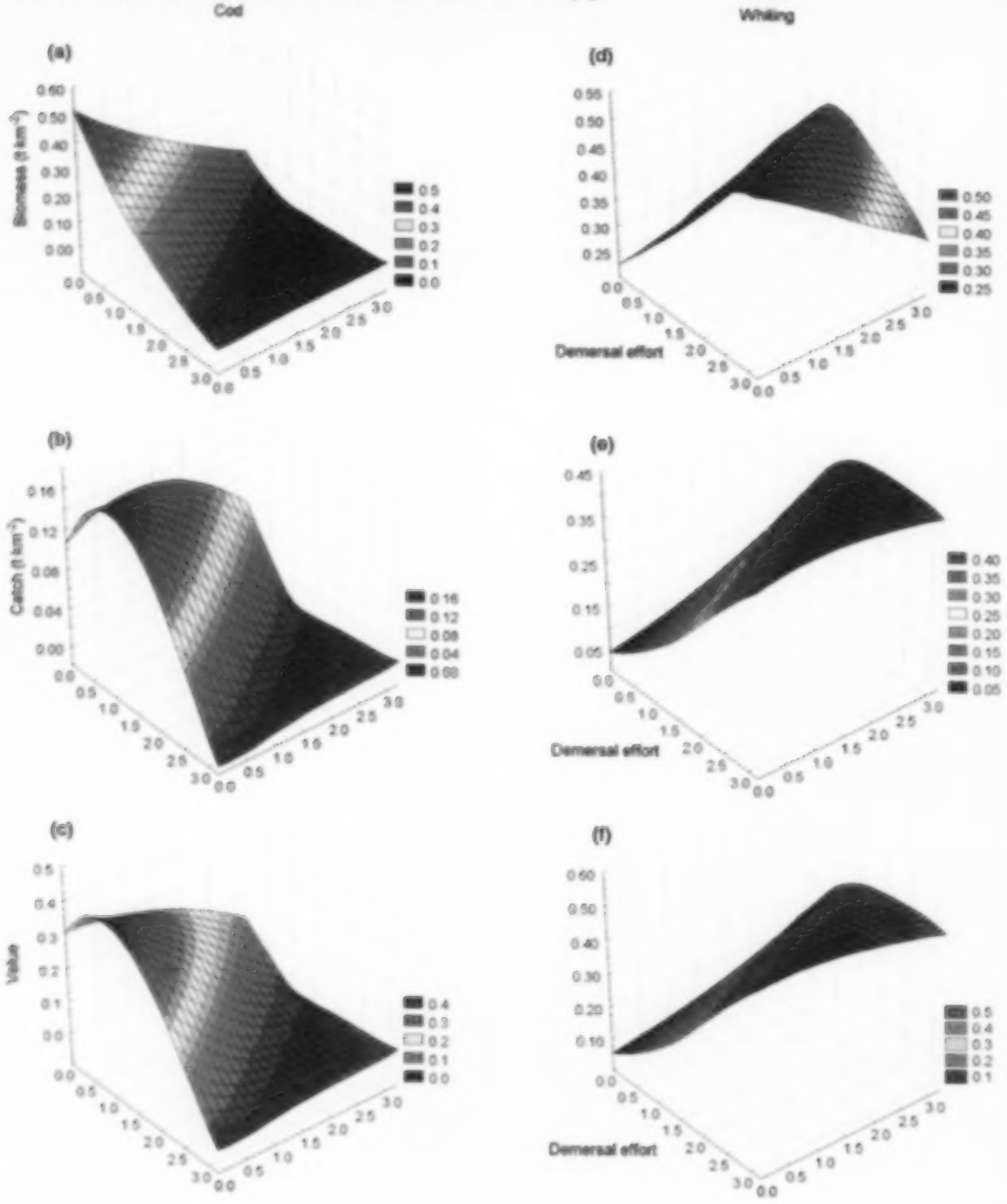
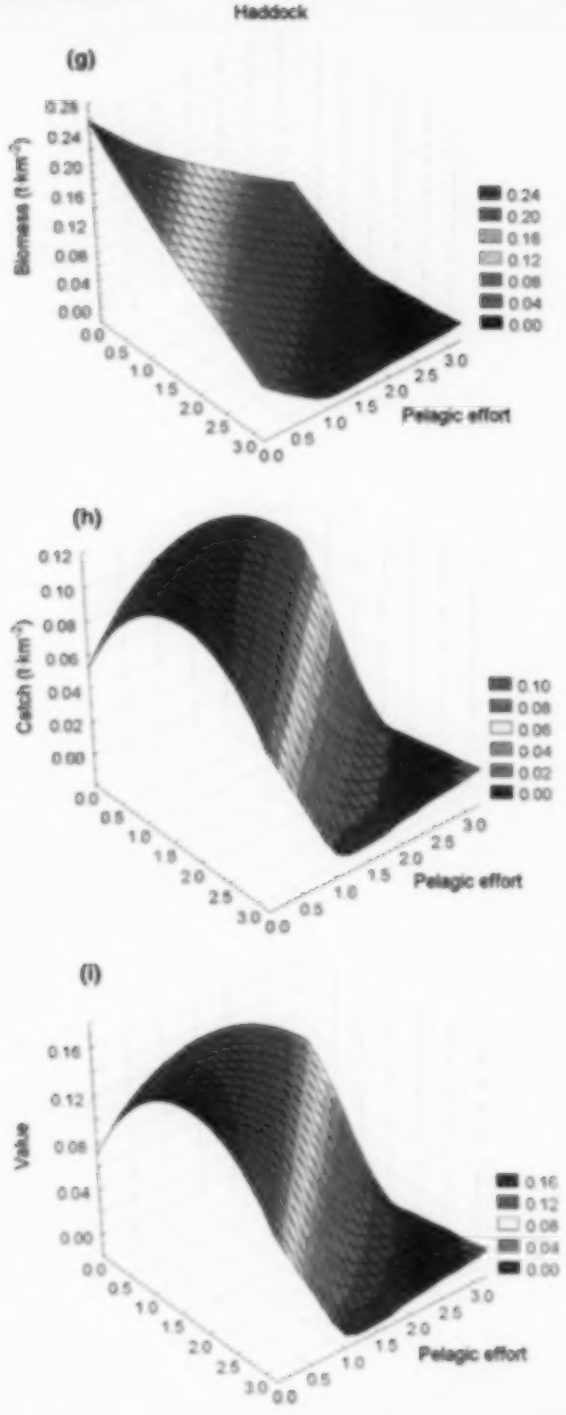


Fig. 6 (*concluded*).



The patterns of response from implementing the predicted F_{MSY} from the ecosystem model are much the same, although whiting biomass is impacted to a larger extent because of the higher-than-baseline fishing mortality. Predicted increases in biomass and catch of cod and haddock are more modest.

Question 3. What happens to yield and revenue in each of the main fisheries, assuming candidate management targets?

We provide a series of three-dimensional response planes that depict changes in the biomass, catch, and value arising from combinations of different levels of effort from demersal and pelagic trawlers (Figs. 6–8). As such, they show the trade-offs between the two fleets. Results are shown for cod, haddock, and whiting separately (Fig. 6), and they are compared with results for the sum of cod, haddock, and whiting (mixed demersals) and the sum of all harvested species in the system (Fig. 7). Results for the two fishing fleets are also shown (Fig. 8).

The effort required to achieve maximum long-term combined yield of cod, haddock, and whiting is different from the effort required to achieve maximum yield of each species alone (Figs. 6*b*, 6*e*, 6*h*, 7*a*). This pattern is true when fleets are considered individually or combined. For example, when the relative effort of pelagic trawlers is maintained at baseline (1), the maximum yield of cod by demersal trawlers is achieved at half the effort (Fig. 6*b*). However, maximum mixed-demersal yield is achieved with a doubling of effort (Fig. 7*b*).

Cod and haddock are compatible in their responses to changes in effort (Figs. 6*a*, 6*g*), the similarity in the overall pattern of long-term yield and value for these species suggesting that a reasonable balance between effort levels can more easily be obtained. It is the contradictory response of whiting that is central to the trade-offs of the mixed-demersal fishery (Fig. 6*d*). With increased effort, whiting biomass increases in response to fishery-induced reductions in cod, its main predator. At higher levels of effort, direct mortality caused by fishing becomes more important than the effect of being released from its predators.

The principal fisheries appear to be at odds with one another, as evidenced by the asymmetric payoffs that result from the changes in species biomasses at alternative effort levels. Catches of demersal trawls are negatively affected in a relatively small way by changes in the relative effort of pelagic trawlers (Fig. 8*a*), indicating that harvesting the prey species (Appendix A, Table A3) is not having a strong influence on the productivity of predators (cod and whiting in particular) through bottom-up effects. It might also indicate that pelagic trawl catches of cod, haddock, and whiting are not large enough to create and impact, though partial *F*s (Appendix A, Table A2) suggest otherwise. Conversely, pelagic trawl fisheries benefit from higher levels of demersal trawl effort (Fig. 8*b*) because of the positive impact that culling of predators (demersal species) has on their prey (pelagic species).

Long-term expectations from a mixed-fishery approach are not commensurate with those of an ecosystem approach; again, there are trade-offs to be made because it is simply not possible to simultaneously be at many different places on the effort response surfaces. Although reduced effort of demersal and pelagic trawl fisheries is beneficial to mixed-demersal stocks and their fisheries (Fig. 7*a*), maximum bio-

Fig. 7. Response curves for mixed-demersal fisheries: (*a*–*c*) cod, whiting, and haddock; and (*d*–*f*) for all groups in the ecosystem (total system).

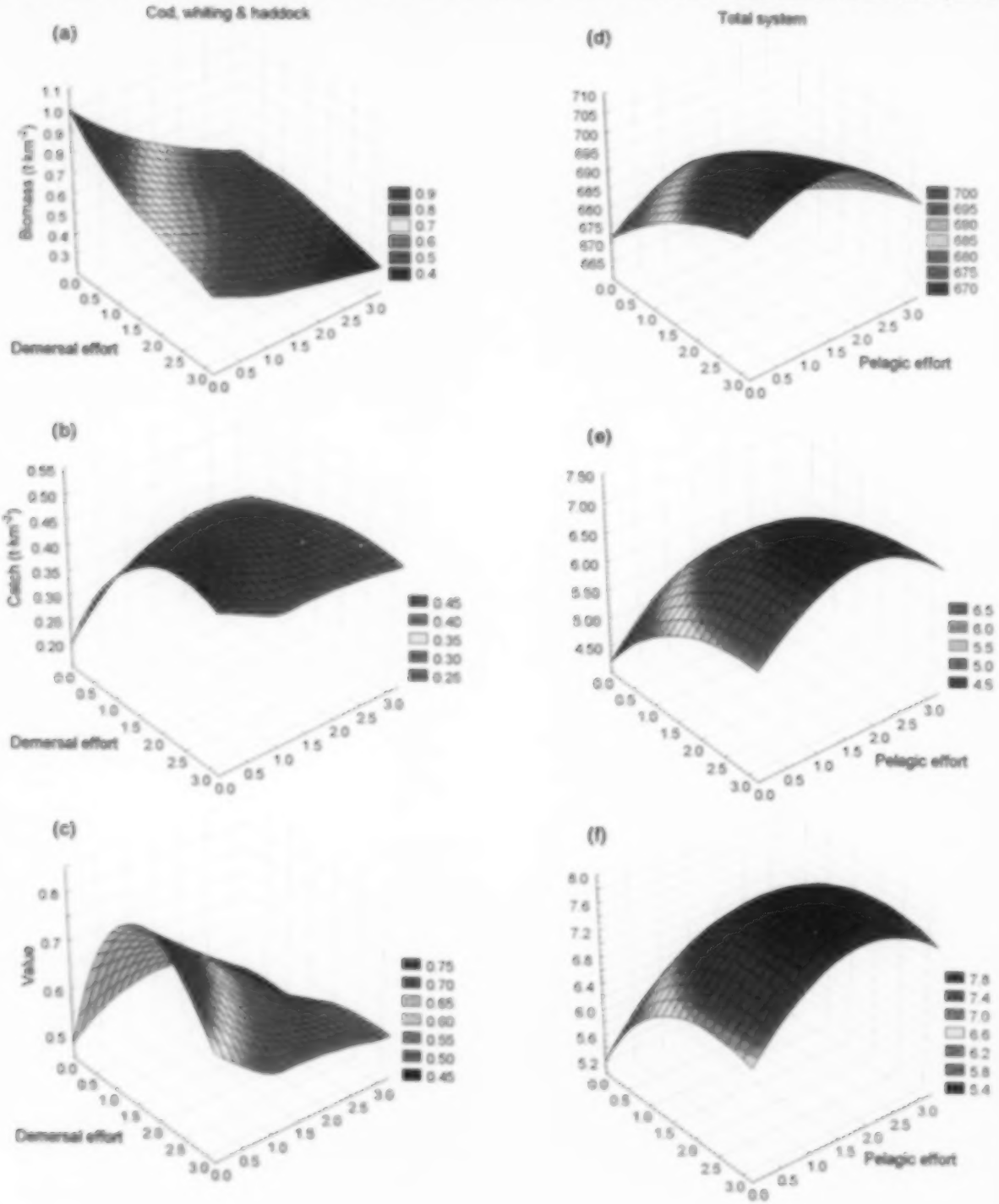
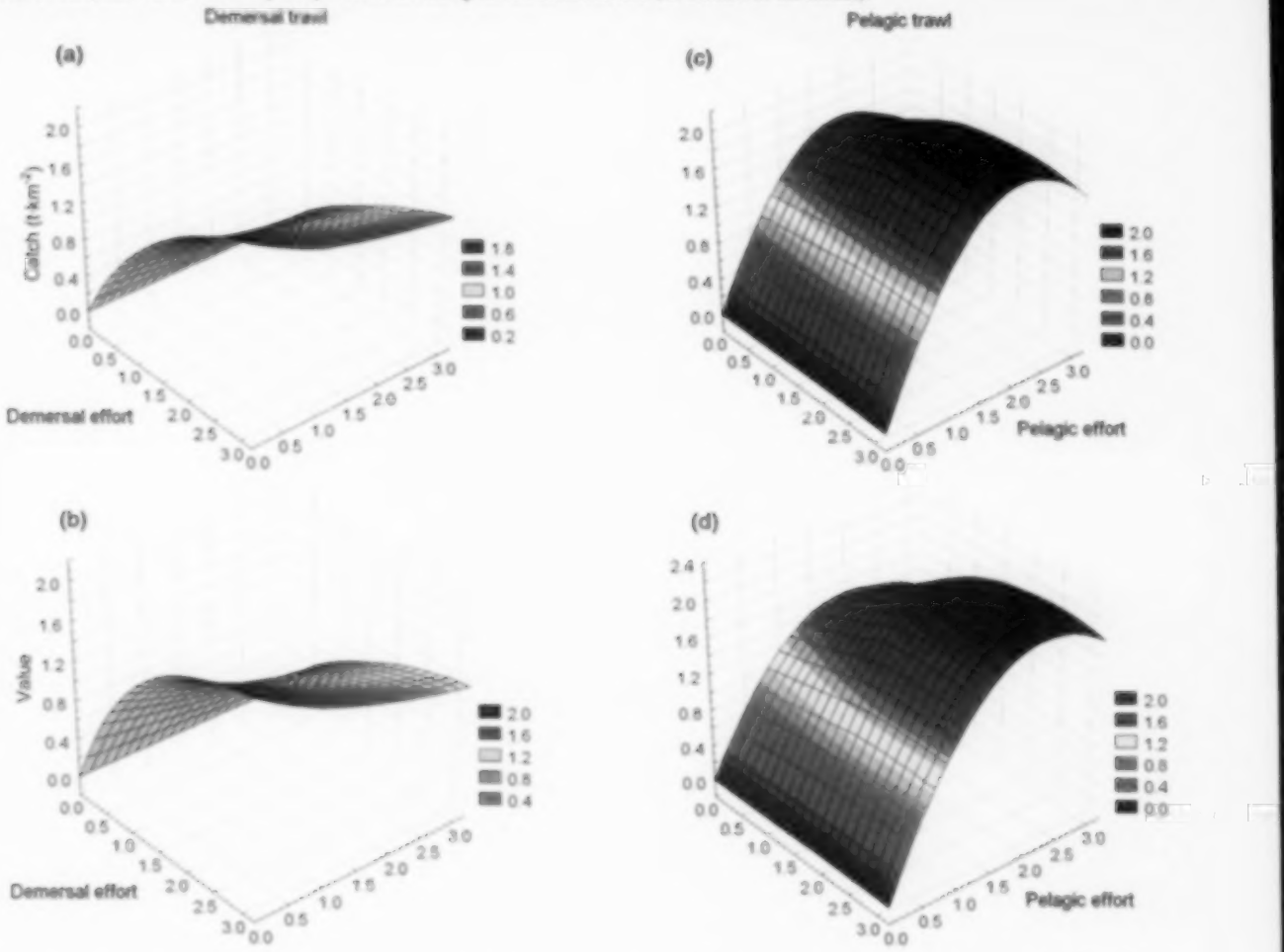


Fig. 8. Response curves for the principal fisheries taking mixed-demersal fish (as defined in the model).



mass, catch, and value from the ecosystem as a whole are, however, obtained at considerably different (higher in this example) levels of fishing effort (Figs. 7*d*, 7*e*, 7*f*). This contrast exemplifies the mixed-fishery or ecosystem conundrum.

Question 4. What happens to the differential responses of cod, haddock, and whiting when environmental effects are included as a driver of the stock dynamics?

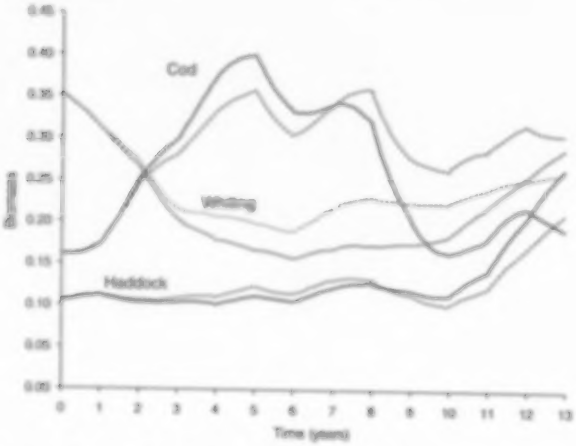
Historical dynamics of cod and whiting appear to be more strongly influenced by environmental factors than haddock (Fig. 9). Time-integrated changes in the biomass of cod and whiting suggest that it is possible that estimates of their productivity may be inflated when environmental influences are not considered. Most certainly, it should be expected that the productivity of fisheries would be different when environmental influence is accounted for (Fig. 10).

Discussion

Predictions from the ecosystem model show that it is not possible to simultaneously achieve the individual MSYs for cod, haddock, and whiting predicted from single-species assessments. In particular, the predicted MSY for whiting is likely to be lower because of the strong predator–prey cross interaction with cod.

Comparisons show that when species interactions are turned on, the ecosystem models' single-species predictions of F_{MSY} are roughly two times higher than F_{MSY} proxies from single-species yield per recruit (Y/R) models. Because the ecosystem model accounts for losses due to predation, the fishing mortality required to achieve MSY is expected to be higher. Past experiences have shown that when Ecosim is fitted to single-species assessment models, predictions can be similar, indicating that the biomass dynamics or delay-difference "submodel" for the target species behaves similarly when "embedded" in Ecosim (with explicit accounting for production and mortality rate as function of food resources and predators) to the corresponding single-species assessment model in which competition effects are represented as implicit functions of stock size (e.g., stock–recruitment model) and predation mortality rates are assumed constant (Christensen et al. 2004). The model used

Fig. 9. Demonstration of the effect of including environmental forcing on the relative dynamics of cod, haddock, and whiting. The plot shows differences in the simulated stock trajectories when changes in primary production are included (solid line) or not (dotted line).

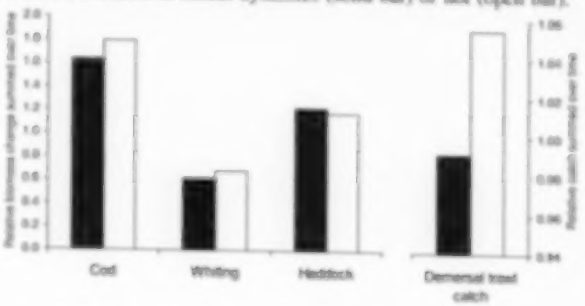


here is fitted to a multispecies model, so it may be expected that predictions of F_{MSY} would be higher than from a single-species model. Such comparisons are difficult to make and should be taken with caution, as the underlying formulations of the models are different.

The simple translation of F_{MSY} to relative fishing effort indicates that MSY of cod, haddock, and whiting is achieved with about 20% less effort for demersal trawlers. However, this analysis is not wholly adequate as it does not account for catches of all species caught by that gear; such a prediction for demersal trawlers indicated that effort would need to be 50% less to achieve MSY for this fleet. Unfortunately, this analysis is also not fully adequate as it does not account for the affects of other gears. A fuller treatment of this problem can only be achieved by considering the response to trade-offs in effort regimes, as is discussed later.

Predictions of the effects of applying proposed EC F_{MSY} proxy fishing rates show also that species differences in response times would make it very difficult to simultaneously achieve biomass targets for each species within the same specified period. Furthermore, because the predicted responses of cod, haddock, and whiting are influenced by both management actions applied to other fisheries and their interactions with other species in the ecosystem, the very notion of MSY based on either a single-species or a mixed-fishery management viewpoint is questionable. This is reasoned from the demonstration that the ecosystem-scale application of F_{MSY} led to wildly different predictions of MSY than either the individual- or mixed-fishery approaches. This result occurs because the fishing mortality on many lower trophic level species (including shellfish) has to be increased substantially to achieve their MSY, the predicted consequences being extensive knock-on effects and severe erosion of ecosystem structure (Walters et al. 2005). Although such ecosystem-scale application of F_{MSY} might be considered extreme, it exemplifies the need to think beyond the cod, haddock, and whiting mixed-fishery problem.

Fig. 10. Comparison of relative changes in biomass of demersal species and catches of demersal trawlers when environmental controls are included in model dynamics (solid bar) or not (open bar).



Cod–whiting interactions and the effects of cannibalism play an important role in predictions of the species and fisheries dynamics and has characteristics that might be capable of producing cultivation–depensation effects that can cause long-lasting unwelcome changes in ecosystem structure (Walters et al. 2000). However, no such dynamics have been observed in the model. Even when cod biomass is very suppressed, it does not appear that whiting are able to "keep cod down", owing to cod dynamics being governed more strongly by top-down effects of fishing on adults and by bottom-up effects on juvenile survival than predation by whiting. Likewise, cod do not appear to be able to suppress recovery of whiting. However, in the investigations performed here, other possible mechanisms that might produce delayed depensation, such as competition–predation trade-offs among species that share similar resources and whose exploitation (or the exploitation of their predators) might give advantage to a competitor that might also be a predator, have not been considered (Yodzis 2001; Walters et al. 2005). For these reasons, Walters et al. (2005) caution that although there is not a lot of evidence for severe disruptions of the natural compensatory responses, possible depensatory effects still represent real risks that need to be included in prudent designs for ecosystem harvest policies.

For the principal fisheries, the combination of effort that produces the highest overall yield and value from mixed-demersal fisheries is not the same as that for individual species. Thus, there exists an incompatibility between the targets for mixed-demersal fisheries and the reference points derived from single-species considerations upon which these are set. The similar responses of cod and haddock to fishing show a congruence that points toward a more "easy deal" being struck for the balance of fishing effort. It is the contradictory response of whiting that is central to the trade-offs in yield and value and thus pivotal in the mixed-demersal fishery conundrum.

Differences in the biomass of each species resulting from different levels of effort have asymmetric pay-offs that put the demersal and pelagic trawl gears at odds with one another (and undoubtedly others too). This effect is tied up with the predator–prey linkages of the target stocks, with pelagic fisheries relying on "prey species" that benefit from the culling of their predators by demersal fisheries. Such prey release and culling phenomena tend to be obvious effects predicted by food web models, particularly when the

links are direct and strong (Pitcher and Cochrane 2002). In the case of the North Sea, with many food web interactions, these effects tend to be more dampened and subtle, but close examination here has revealed the importance to the mixed-fishery problem. Walters et al. (2005) observed similar asymmetries in 11 ecosystems historically subject to high fishing pressure; predators were influenced more by top-down effects of fishing than by bottom-up effects of fishing on their prey.

Differences in how species respond to the effects of climate change indicate that the development of management plans should not be based solely on knowledge of responses to fishing impacts. In the simple illustration given, there were differences in how cod, haddock, and whiting were affected by environmental change (either directly or indirectly), and this had an important bearing on expected catches for demersal fisheries. Taking account of the effect of environmental change and fishing on species dynamics and determining their relative influence is challenging research of vital importance to developing robust long-term fisheries management plans (Mackinson et al. 2008; Perry et al. 2009).

This contribution marks but a small piece in the NSRAC's puzzle to develop long-term management plans. That mixed-fishery and ecosystem-scale considerations give rise to contrary predictions for the best "balance" of fishing effort for mixed-demersal fisheries exemplifies the difficult challenges faced when moving toward an ecosystem approach. The general result that MSY targets for species, assemblages, and the ecosystem are not mutually compatible points to the need to move towards an analysis of trade-offs involving optimization methods conditioned on defined goals for fishery management (e.g., Zabel et al. 2003; Christensen and Walters 2004*b*; Araújo et al. 2008). Such an exercise is best achieved as part a decision analysis process, and so the logical next step for this work is to use the knowledge from the current analysis to help the NSRAC formulate plausible management scenarios and examine the trade-offs that are embedded in the layers of ecology and decision-making. Throughout such a process, a key challenge for those involved in using complex ecosystem and multispecies models to explore harvest strategies is distilling the information (and uncertainties) into a readily communicable form. Without doing so, the knowledge will not be able to contribute effectively to decision-making (Pope 1997). The work of Collie et al. (2003) on using amoeba diagrams to synthesize multispecies advice and work on risk analysis in management strategy evaluation for North Sea fisheries (e.g., Kell and Fromentin 2006) are of particular interest here. Colour-coded summaries (e.g., Caddy 2002; Mackinson et al. 2008) and simple decision tables appear to be some of the more accessible approaches that should be considered.

Driven by the need to assess how food web interactions, habitat effects, and responses to fishing and climate change influence species dynamics and fisheries, multispecies and ecosystem models are becoming increasingly refined (see Plagányi (2007) and ICES (2007)). However, few are currently used to implement management decisions (Pinnegar et al. 2008), principally because of large uncertainties associated with model formulations, structure, parameters, and outputs. Some of the uncertainties of model predictions arising from data and parameter uncertainty can be accounted for, but this "safeguard" does not negate the fact that predictions might simply be wrong because of bad estimates or errors of omission for a few key parameters and processes. Although it has to be accepted that it is necessary to make trade-offs associated with the level of detail in accounting for processes in time and space and availability of data, it is important to be ever vigilant and critical in the use of such models. The ecosystem model used here is able to account for complex cross-species and cross-fishery interactions necessary to address the questions, but there are several uncertainties and necessary model assumptions that influence the results and their interpretation. Principal among these are the quality of diet data and specification of vulnerability parameters underpinning formulation of the foraging arena concept of predator–prey interactions in Ecosim (see Walters et al. (1997) and Walters and Martell (2004)), as together these define the predator–prey interactions for all species in the model.

Diets of fish are known to vary widely in space and time, so the model inherits the burden of uncertainty already carried by the "1991 Year of the Stomach" data, which are, nevertheless, the best information available for the North Sea (in terms of its comprehensive coverage and number of samples). Somewhat contrary to the data used in the model, recent diet investigations responding to concerns over the impact of whiting predation on depressed cod stocks suggest a very low incidence of predation on cod by whiting, a result that is partly conditioned on the very low availability of juvenile cod as a prey (Stafford et al. 2007).

Parameterisation of vulnerabilities through calibration of the model to alternative data sets from survey data and single-species models rather than MSVPA (although single species vary little from MSVPA) would yield somewhat different vulnerability parameters. Such analyses are not trivial and are planned for future investigations.

Results of the trade-off among gears are affected by the fact that the model is not able to account for uncertainty associated with changes in targeting of the fleets. The model assumes stability in the targeting of species (which is defined by the partial mortalities derived from STCF 1991 catch composition; Vinther and Thomsen 1992), with catches varying in response to changes in relative biomass target species. Finally, it is worth pointing out that the model is not able to find technical solutions to the mixed-fishery problem such as might arise through consideration of fine-scale differences in the spatial and temporal behaviour of cod, haddock, and whiting combined with innovation in fishing operations.

To end on a more positive note, even though few multispecies and ecosystem models (or applications thereof) have yet met the "standards" expected for routine use, this investigation of the complex dynamics of the North Sea mixed fishery for cod, haddock, and whiting shows that their continued development is essential in gaining a more comprehensive understanding of ecosystems and fishery dynamics. With a little luck, ecosystem models might at least help point us in the right direction or, better perhaps, tell us when we heading in the wrong one.

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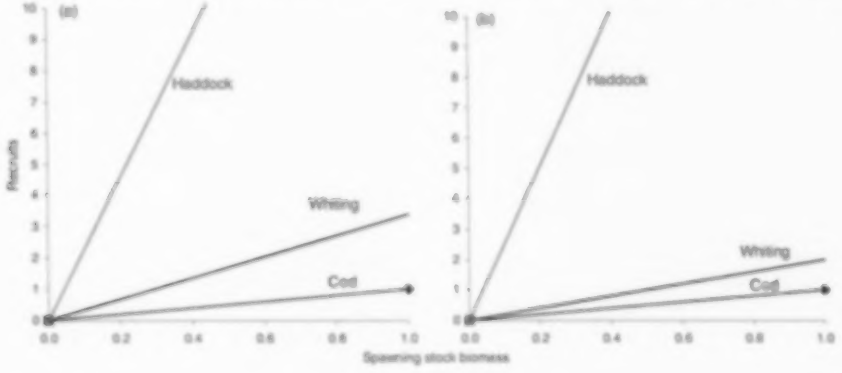
Appendix A. Characterising the species and fishery interactions

Compensatory response

Examination of stock–recruitment relationships from single-species and multispecies models shows that haddock and whiting have historically exhibited relatively stronger compensatory responses than cod (Fig. A1). The steeper lines for haddock are characteristic of the sporadically high recruitment events that lead to dominant year classes in the fishery.

Fig. A1. Comparison of the relative degree of compensatory response among North Sea demersal fish. Lines indicate the slope of the 50 percentile *R*/SSB relative to that of cod (set to 1): (*a*) data from the MSVPA 2005 key run; (*b*) data from single-species assessments.



Predator–prey interactions

Mortality sources and interactions among cod, haddock, and whiting and their main predators and prey are given (Tables A1, A2, A3) and briefly summarized below, supplemented with information from the ICES Study Group on Multispecies Assessment in the North Sea (ICES 2005). Juvenile cod are preyed upon by a large variety of species. Estimations of predation impacts indicate that gurnards, horse mackerel (when present), squids, seals, and seabirds can be important predators relative to whiting and adult cod. Fisheries account for the largest mortality of adult cod, whereas seals are the major source of their predation mortality. For whiting age 0–1, cannibalism by adults and consumption by horse mackerel, cod, and grey gurnard are important sources of predation mortality, and for ages 1–2, cod becomes the dominant predator. Fishing mortality accounts for the largest source of mortality for whiting age 3 and older, with seals having the largest predation impact. The

Table A1. Biomass, rate parameters, and predation mortality sources used in the ecosystem model.

	Cod		Whiting		Haddock	
Group number	Juveniles (0–2, 0–40)	Adults	Juveniles (0–1, 0–20)	Adults	Juveniles (0–1, 0–20)	Adults
Biomass (tonnes)	45 030	91 770	126 540	200 640	161 880	59 280
Production/biomass (year^{-1})	1.79	1.19	2.36	0.89	2	1.14
Consumption/biomass (year^{-1})	5.96	3.5	6.58	5.46	5.39	4.4
Predation mortalities by predators						
Baleen whales	—	—	0.0714	—	—	—
Toothed whales	0.0744	—	**0.2641**	**0.0427**	0.027	**0.0084**
Seals	**0.0815**	**0.0806**	0.0473	**0.0399**	0.0048	**0.0231**
Seabirds	0.0255	—	0.0253	—	0.0251	—
Juvenile sharks	—	—	0.0001	0.0001	—	—
Spurdog	—	—	0.0042	0.0107	0.0007	0.0019
Small sharks	—	—	—	0.0001	—	—
Juvenile rays	0.0115	—	—	—	—	—
Starry ray and others	0.0631	—	0.0263	0.0023	0.0085	—
Thornback and spotted ray	—	—	0.0122	—	0.0017	—
Skate and cuckoo ray	—	—	0.006	—	0.0017	—
Juvenile cod (0–2, 0–40 cm)	0.0298	—	0.0492	—	0.04	—
Cod (adult)	0.0606	**0.035**	**0.1185**	**0.1455**	0.0405	**0.1046**
Juvenile whiting (0–1, 0–20 cm)	0.0684	—	0.0454	—	**0.0453**	—
Whiting (adult)	0.0462	—	**0.4221**	0.0081	**0.0993**	—
Haddock (adult)	0.0006	—	—	—	0.0172	—
Juvenile saithe (0–3, 0–40 cm)	—	—	0.0432	—	0.0117	—
Saithe (adult)	—	—	0.0143	**0.0441**	0.0438	**0.0152**
Hake	—	—	0.0041	—	—	—
Other gadoids (large)	0.039	—	0.0278	—	0.0019	—
Other gadoids (small)	—	—	0.0181	—	—	—
Monkfish	0.0091	**0.0045**	0.0329	**0.0207**	0.0145	**0.0395**
Gurnards	**0.0814**	—	**0.0969**	—	0.0059	—
Mackerel	0.0038	—	0.0013	—	—	—
Horse mackerel	**0.1312**	—	**0.2828**	—	**0.1474**	—
Long-rough dab	—	—	0.0249	—	—	—
Turbot and brill	**0.0794**	—	0.0668	—	—	—
Halibut	—	—	**0.1404**	—	**0.0882**	—
Squid and cuttlefish	**0.2111**	—	0.0751	—	**0.0587**	—

Note: Diet composition data from the 1991 stomach sampling programme (Hislop et al. 1997) together with other specific diet studies are used in calculating the predation mortalities. The top five for each species are indicated by bold type. Numbers in parentheses after juveniles represent age (in years) and length (in cm), respectively.

main diet of whiting is commercially important fish species, including whiting, sprat, Norway pout, sandeel, and juvenile haddock and cod. Juvenile haddock are an important prey for whiting and horse mackerel, whereas cod, monkfish, seals, and saithe are key fish predators of adult haddock. Once again, fisheries account for the largest part of the total mortality of adult haddock. In contrast to whiting and cod, haddock eats few fish, with its diet consisting mostly of the benthic and epibenthic invertebrates. Cross-predation impacts among cod, haddock, and whiting are given (Table A3).

Fishery interactions

Estimated fleet-specific sources of fishing mortality (partial *F*s) on North Sea stocks of cod, haddock, and whiting in the base year of the model (1991), together with the relative contribution of each fleet to the overall *F* on each species, are presented (Table A4). Partial *F*s are calculated according to the catch–biomass ratio, where catch data are taken from STCF 1991 and biomass are derived from the MSVPA 2005 key run (ICES 2005). The *C*–*B* ratios are a reasonable approximation of the overall estimated *F*s calculated by MSVPA (see Table 2). Catch composition of the principal gears for mixed demersal fisheries (based on their contribution to mortality of each species) are shown (Table A5).

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Table A2. Comparison of estimated of mortality sources for cod, haddock and whiting.

	1991 (base year)					More recent estimates (2003–2005)				
	M1	*M2*	*M*	*F*	*Z*	*M1*	*M2*	*M*	*F*	*Z*
Ecosystem model										
Cod (adult)	0.30	0.12	0.42	0.77	1.19					
Juvenile cod (0–2 years, 0–40 cm)	0.11	1.02	1.13	0.66	1.79					
Whiting (adult)	0.06	0.31	0.37	0.52	0.89					
Juvenile whiting (0–1 years, 0–20 cm)	0.33	1.92	2.25	0.11	2.36					
Haddock (adult)	0.03	0.19	0.22	0.92	1.14					
Juvenile haddock (0–1years, 0–20 cm)	1.09	0.68	1.78	0.22	2.00					
MSVPA										
Cod (2–8 years)	0.20	0.09	0.28	0.82	1.10	0.20	0.11	0.31	0.91	1.21
Juvenile cod	0.20	1.02	1.22	0.16	1.38	0.20	1.64	1.84	0.11	1.95
Whiting (2–6 years)	0.20	0.24	0.44	0.62	1.06	0.20	0.29	0.49	0.15	0.65
Juvenile whiting	0.20	1.17	1.37	0.13	1.50	0.20	1.91	2.11	0.14	2.25
Haddock (2–6 years)	0.20	0.08	0.28	0.80	1.08	0.20	0.09	0.29	0.20	0.49
Juvenile haddock	0.20	1.21	1.41	0.16	1.56	0.20	1.91	2.11	0.10	2.21
Single-species assessments										
Cod (2–4 years)			0.27	0.89	1.15			0.27	0.86	1.13
Whiting (2–6 years)			0.32	0.74	1.06			0.32	0.25	0.57
Haddock (2–4 years)			0.30	0.94	1.24			0.30	0.32	0.62

Note: Values are given for 1991 (the base year of the ecosystem model used in this investigation) and more recent estimates from single-species assessments and the 2005 key run of MSVPA (ICES 2005). *M1*, other mortality; *M2*, predation mortality; *M*, total natural mortality; *F*, fishing mortality; *Z*, total mortality.

Table A3. Percentage of interspecies predation mortality among cod, haddock, and whiting used in the model (values used in the model are derived from the 1991 stomach sampling program (Hislop et al. 1997)).

	Predator						
Prey	Juvenile cod (0–2, 0–40), %	Cod (adult), %	Juvenile whiting (0–1, 0–20), %	Whiting (adult), %	Juvenile haddock (0–1, 0–20), %	Haddock (adult), %	Total, %
Juvenile cod (0–2, 0–40)	3	6	7	5	0	0	20
Cod (adult)		29			0		29
Juvenile whiting (0–1, 0–20)	3	6	2	22	0		33
Whiting (adult)		46		3	0		49
Juvenile haddock (0–1, 0–20)	6	6	7	1	0	3	35
Haddock (adult)		54			0		54

Note: Numbers in parentheses after juveniles represent age (in years) and length (in cm), respectively.

Table A4. Comparison of fishing mortality sources for cod, haddock, and whiting used in the model (proportional estimates determined from catch composition data (1991 STCF database)). Values shown in *B* are the percentage contribution by each fleet to the total mortality of each species.

(*A*) Partial *F* values.

Group name	Demersal trawl + dem seine	Beam trawl	Sandeel trawl	Pelagic trawl	Drift and fixed nets	Nephrops trawl	Other	Total catch (t)	Biomass (t)	*F* = *C*/*B* base year
Cod (adult)	0.40	0.06	0.01	0.17	0.12	0.01	0.00	70 794	91 770	0.77
Whiting (adult)	0.18	0.01	0.16	0.14	0.00	0.01	0.01	103 455	200 640	0.52
Haddock (adult)	0.36	0.00	0.08	0.35	0.00	0.03	0.09	54 264	59 280	0.92

(*B*) Proportional *F* values for each gear.

Group name	Demersal trawl + dem seine (%)	Beam trawl (%)	Sandeel trawl (%)	Pelagic trawl (%)	Drift and fixed nets (%)	Nephrops trawl (%)	Other (%)	Total catch (t)	Biomass (t)	*F* = *C*/*B* base year
Cod (adult)	52	8	2	22	15	1	0	70 794	91 770	0.77
Whiting (adult)	35	3	31	27	0	2	2	103 455	200 640	0.52
Haddock (adult)	39	0	8	39	0	3	10	54 264	59 280	0.92

Table A5. Main components of the catch for principal fisheries responsible for the majority of mortality on cod, haddock, and whiting.

	Demersal trawl + demersal seine (%)	Pelagic trawl (%)
Cod	8.4	2.4
Whiting	9.2	4.7
Haddock	9.1	4.7
Saithe	11.9	2.0
Other gadoids and demersals	12.0	0.8
Herring	12.0	29.7
Blue whiting	0.0	4.8
Norway pout	0.0	9.6
Mackerel	2.1	35.9
Plaice	9.2	0.2
Dab	15.9	0.0

Note: Values are percentage of the total catch by weight (see Table 2 for fishing mortalities).

Appendix B. Fitting the model simulations to time series data (MSVPA 2005 key run) and tuning compensation in stock–recruitment relationships

An account of the testing of the basic model dynamics, strategy, and methodological approach for parameterising Ecosim through time-series fitting is provided in Mackinson and Daskalov (2007). The purpose of this account is to provide readers with sufficient information to understand the fitting procedure and analysis of stock–recruitment used to specify the relative degree of compensation.

The model was calibrated to outputs from MSVPA for two reasons: (*i*) it makes more sense than fitting to output from single-species models that do not account for species interactions; and (*ii*) because MSVPA is an accepted standard for multispecies modelling in the North Sea and credibility of the results and expectations of the ecosystem model are (rightly or wrongly) judged by others with this in mind. MSVPA was not used in the investigation because initial screening of candidate models indicated that EwE was a more appropriate modelling tool for addressing the long-term strategic ecosystem-scale questions considered here.

General procedure for fitting model simulations to the MSVPA 2005 key run time-series data

Time-series fitting (1991–2004) was used estimate the vulnerability (v) of each prey species to its predators. Prey vulnerabilities represent the rate of exchange (flow control) of biomass between two prey behavioural states: a state vulnerable to predation and a state invulnerable to predation. The rationale of this representation is that at a given moment in time, not all prey biomass is available to predators; predator–prey relationships in nature are often limited by behavioural and physical mechanisms, for example, the schooling behaviour and diel vertical migrations of clupeid fish and spatial refuges used by many reef fish that considerably limit exposure to predation (Walters and Martell 2004).

The fitting process was a lengthy iterative procedure that used nonlinear optimization algorithms to improve the goodness of fit between model predictions and observed data by making adjustments mainly to the vulnerability parameters. During time-series fitting, data from MSVPA and other sources were used for two purposes: (*i*) to drive the changes in the model by forcing of fishing mortality and changes in primary production; and (*ii*) to provide a history of the changes in the biomass of species (absolute or relative) against which the models predictions were compared for each parameterisation (Fig. B1).

In addition to the prey vulnerabilities, Ecosim requires input parameters that define linkages between multistanza groups (adult and juveniles) and a parameter that represents the behavioural trade-off associated with feeding and exposure to predation risk. In combination, these influence the degree of compensation in recruitment and are an integral part of the parameterisation procedure required to fit simulations to time-series data. To ascertain the relative between-species differences in the degree of compensation that the model needed to represent, an analysis of stock–recruitment data was undertaken (see below).

Analysis of stock–recruitment relationships

Compensatory responses are measured through stock–recruitment relationships, with stronger compensation being typified by "curves" that rise more steeply from the origin before flattening off.

Stock–recruitment data for cod, haddock, whiting, herring, and saithe (the multistanza groups specified in the model) were obtained from MSVPA 2005 key run and single-species stock assessments. The data were lagged by appropriate year (1 for cod, whiting, and herring; 3 for saithe; 0 for haddock) and plotted. Based on the rationale of F_{msy} calculations (Sissenwine and Shepherd 1987), the 50 percentile R/SSB was calculated and plotted (50% of the SSB, and R data points are above the line and 50% are below) (Fig. B2). Using cod as a reference species, comparison was made between the relative slopes of the 50% R/SSB lines for the purpose of tuning the relative degree of compensation for each multispecies group in the model (see Appendix A, Fig. A1).

Fig. B1. Comparison of ecosystem model simulations (solid line) with biomass and yield estimates taken from MSVPA (2005 key run) (dotted lines with points). MSVPA yield estimates were not available for mackerel and horse mackerel. (Figure B1 continues on pages 1126–1128.)

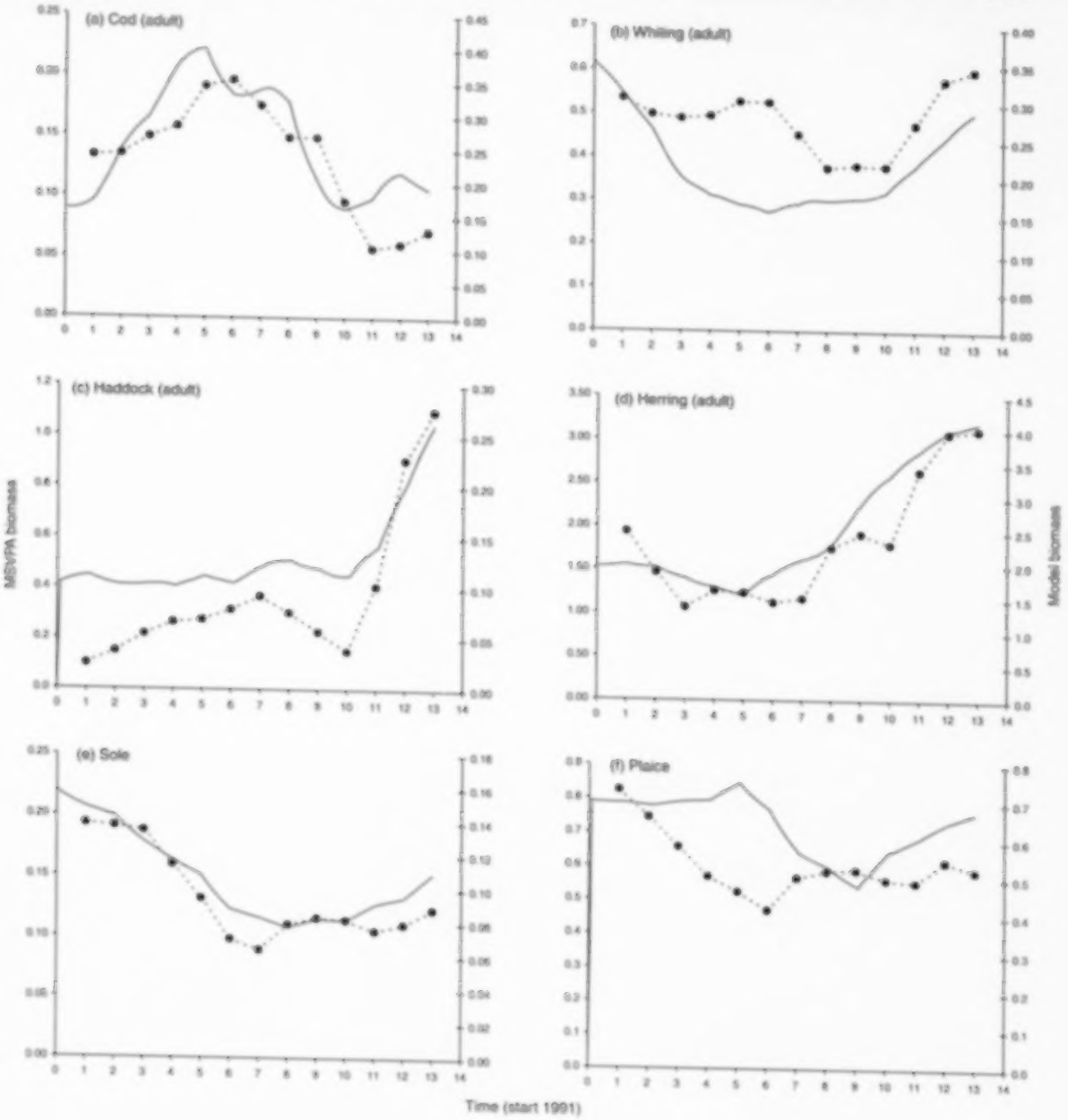


Fig. B1 *(continued).*

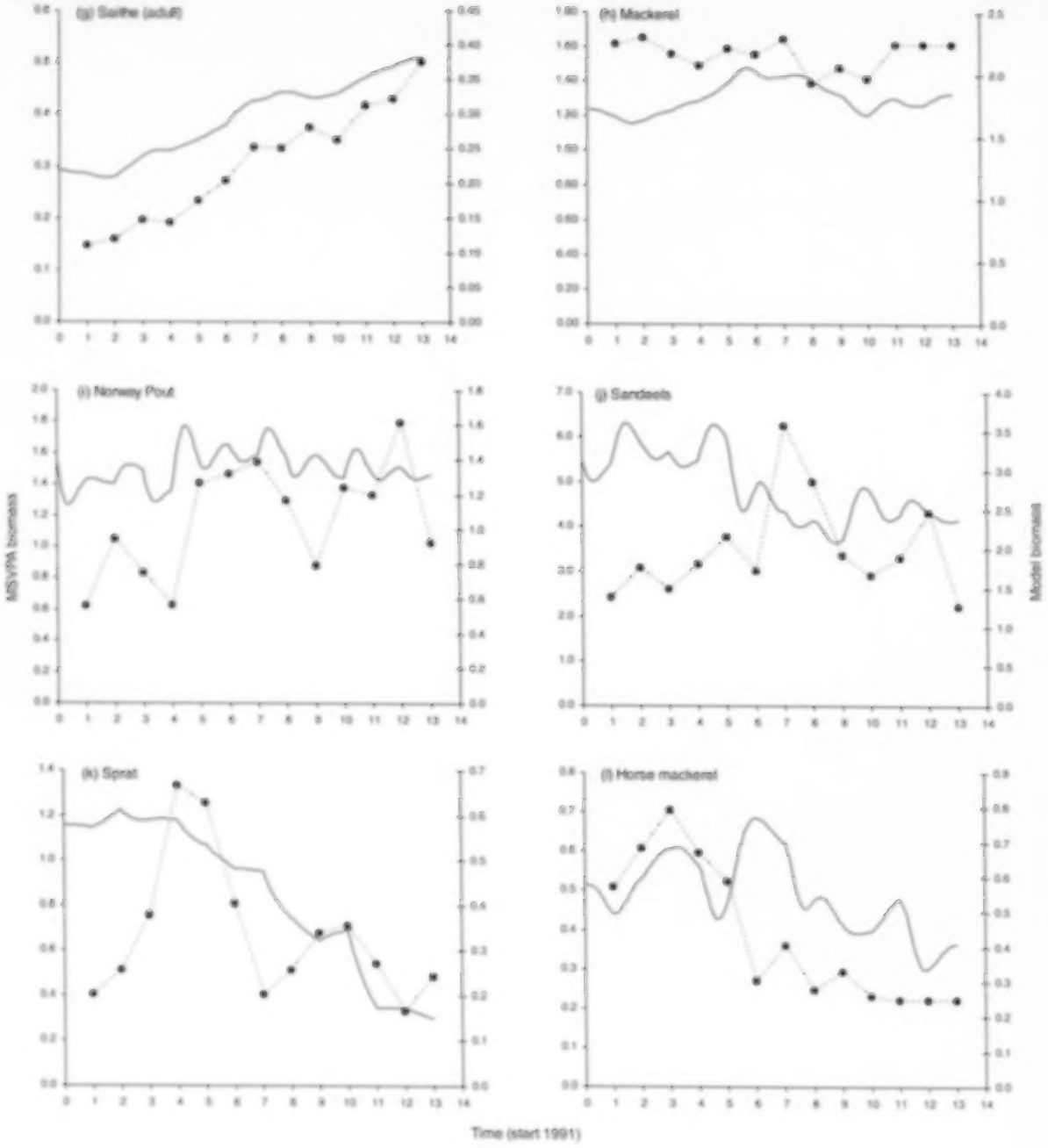


Fig. B1 *(continued).*

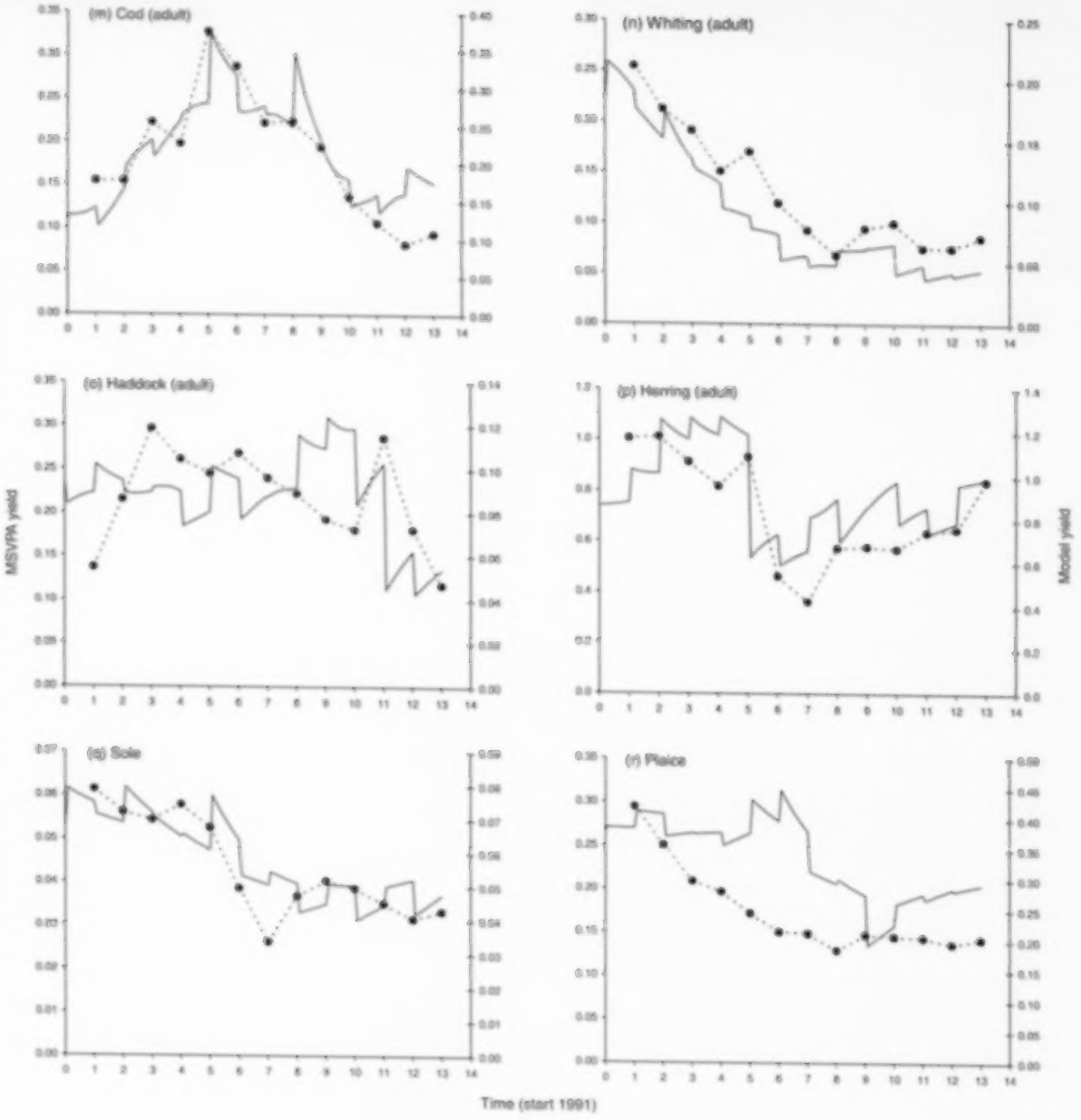


Fig. B1 (*concluded*).

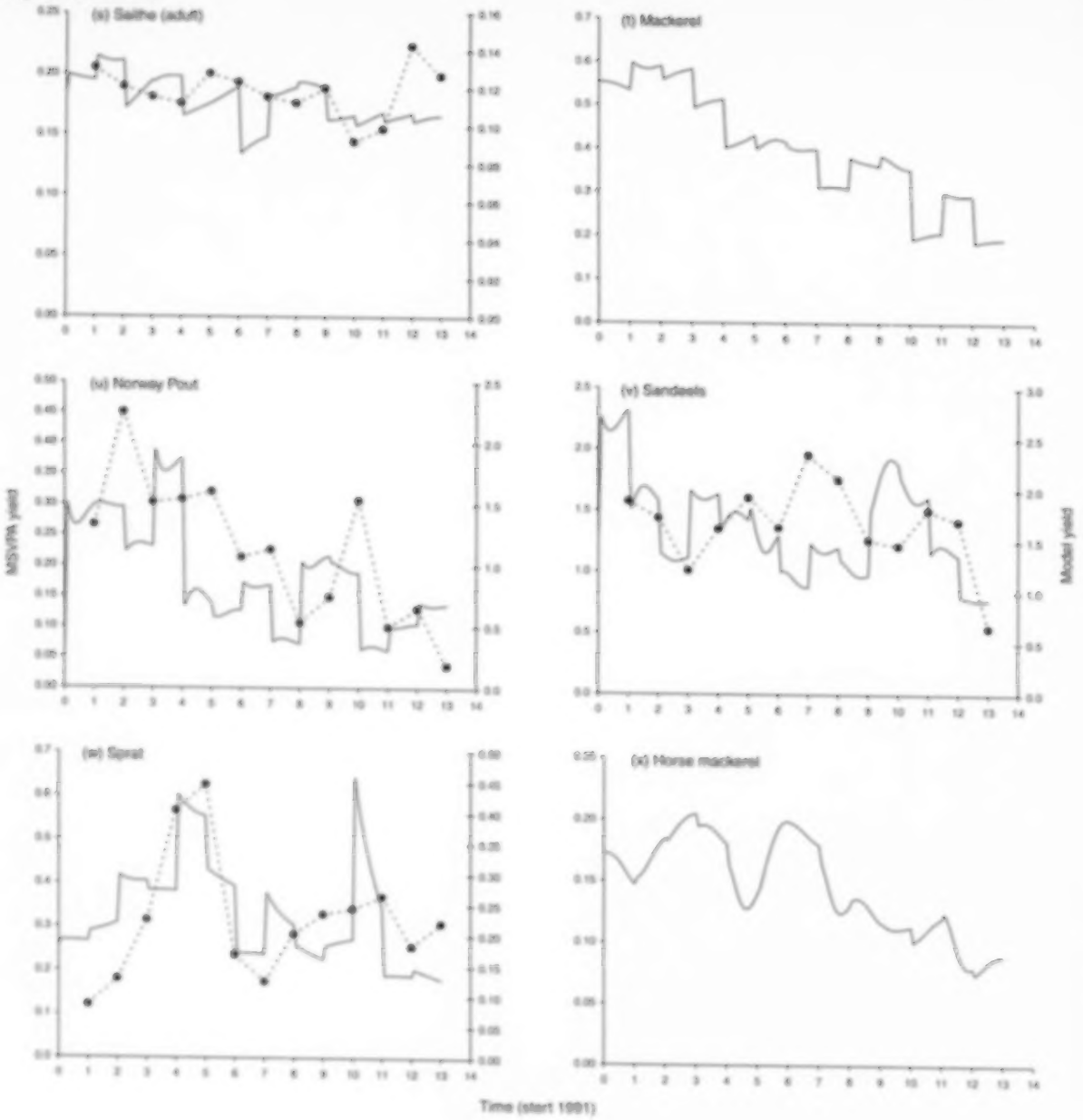
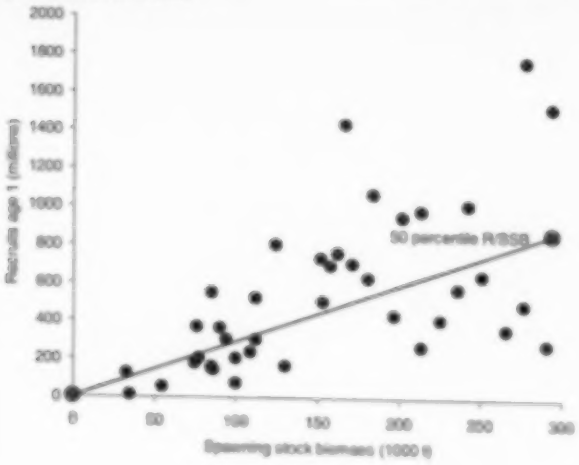


Fig. B2. Stock–recruitment plot for cod MSVPA data with the 50 percentile *R*/SSB line.



Lead–radium dating of orange roughy (*Hoplostethus atlanticus*): validation of a centenarian life span

Allen H. Andrews, Dianne M. Tracey, and Matthew R. Dunn

Abstract: Life-span estimates for orange roughy (*Hoplostethus atlanticus*) range from ~20 years to well over 100 years. In this study, an improved lead–radium dating technique provided independent age estimates from sagittal otoliths. This technique used the known properties of radioactivity for lead-210 and radium-226 to determine the validity of fish age estimates. An improvement to lead–radium dating using mass spectrometry allowed the use of smaller samples than previously possible; therefore, an application was made to otolith cores, the first few years of otolith growth. This approach circumvented the use of whole otoliths and alleviated many of the assumptions that were necessary in previous lead–radium dating applications. Hence, it was possible to critically evaluate lead–radium dating as a tool in fish age validation. The measurement of lead–radium ratios for a series of age groups that consisted of otolith cores, grouped based on growth-zone counts from thin sections, showed a high degree of correlation to the expected lead–radium ingrowth curve. This finding provided support for age estimation procedures using thin otolith sectioning. As independent estimates of age, the results indicated that fish in the oldest age group were at least 93 years old, providing robust support for a centenarian life span.

Résumé : Les estimations de la longévité de l'hoplostèthe orange (*Hoplostethus atlanticus*) varient de ~20 ans à nettement plus d'un siècle. Dans notre étude, une méthode améliorée de détermination de l'âge basée sur le plomb et le radium fournit des estimations de l'âge indépendantes à partir des otolithes sagittaux. La technique utilise les propriétés connues de radioactivité du plomb-210 et du radium-226 pour déterminer la validité des estimations de l'âge chez les poissons. Une amélioration de la détermination de l'âge basée sur le plomb et le radium par l'utilisation de la spectrométrie de masse permet l'analyse d'échantillons plus petits qu'il n'était possible antérieurement; c'est pourquoi nous l'avons appliquée aux noyaux des otolithes qui représentent les premières années de croissance des otolithes. Cette méthodologie évite l'utilisation d'otolithes entiers et atténue plusieurs des présuppositions antérieurement nécessaires pour la détermination de l'âge à l'aide du plomb–radium. Il est ainsi possible d'évaluer de manière critique la détermination de l'âge par le plomb–radium comme outil de validation de l'âge chez les poissons. Les mesures des rapports plomb–radium pour une série de classes d'âge représentées par des noyaux d'otolithes regroupés d'après le nombre de zones de croissance sur des coupes minces, présentent une forte corrélation avec la courbe attendue de recrutement plomb–radium. Cette observation vient valider les procédures d'estimation de l'âge basées sur les coupes minces d'otolithes. En tant qu'estimations indépendantes de l'âge, nos résultats indiquent que les poissons appartenant au groupe le plus âgé ont au moins 93 ans, ce qui appuie solidement l'hypothèse d'une longévité d'un siècle.

[Traduit par la Rédaction]

Introduction

Orange roughy (*Hoplostethus atlanticus*, family Trachichthyidae) is widely distributed at depths of 600–1400 m in most temperate oceans and is a common target species for deepwater trawl fisheries around the world. The development of deepwater fisheries has been relatively recent, with the first major orange roughy fisheries developing off New Zealand in the late 1970s, followed by fisheries off Australia in the mid-1980s, smaller fisheries in the northeastern Atlantic Ocean in the early 1990s, and fisheries off Namibia, Chile, and the southern Indian Ocean through the mid- to late 1990s (Bax et al. 2005; Japp and James 2005; Large and Bergstad 2005). Orange roughy fisheries have proven difficult to manage sustainably; many of these areas have supported boom-and-bust fisheries, with much of the catch

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occurring in the first few years, followed by a period of substantial catch rate declines, and culminating in fishery closures or abandonment (Branch 2001; Clark et al. 2007; Sissenwine and Mace 2007).

The most well developed and productive orange roughy fisheries in the world have been from the waters off New Zealand (Branch 2001). Of these, the largest fishery has been situated on the Chatham Rise, where annual catch levels of around 40 000 tonnes (t) were reached in the late 1980s. However, by 2007–2008, these were reduced to about 10 500 t following a series of total allowable commercial catch (TACC) reductions, and sustainability at this level has been a growing concern (Ministry of Fisheries 2008). A similar scenario was realized for orange roughy fisheries off Australia, where catch quotas were reduced over time (Bax et al. 2005); in 2006, orange roughy were declared threatened by the Australian Department of Environment and Heritage. An approach perceived as conservative was applied to recent seamount fisheries off Namibia with the aim of establishing sustainability. The fisheries appeared to have suffered quickly from overexploitation (Boyer et al. 2001; Butterworth and Brandão 2005); however, an intermittent spawning aggregation is being considered as a hypothesis for the observed changes in the fishery (McAllister and Kirchner 2001; Oelofsen and Staby 2005).

Although major efforts have gone into managing orange roughy fisheries, after three decades of exploitation, almost all stocks have been fished down substantially, and concerns are raised regularly about whether sustainability is attainable (Clark et al. 2007; Sissenwine and Mace 2007). High vulnerability to fishing pressure combined with rapid development of the fishery led to a substantial catch before management was introduced (Clark 2001; Lack et al. 2003; Morato and Clark 2007). When the accumulated biomass of a stock has been fished down, the sustainability of the fishery will be dependent on stock productivity. The productivity of orange roughy stocks is believed to be especially low but remains poorly understood (Francis and Clark 2005; Sissenwine and Mace 2007).

In most stock assessments, productivity is determined using ageing analyses to estimate growth, age at maturity, longevity, rate of natural mortality, and recruitment variability. Although there is growing confidence in current age interpretations of deepwater fish species and in proposed mechanisms for how some may attain high longevity (Cailliet et al. 2001), many age estimates are based solely on sagittal otolith growth zone counts. In some cases, the lack of a convincing age validation associated with these counts has lead to disbelief in the age interpretations; the orange roughy, believed to live to ages over 100 years, has been one of the most debated species (Gauldie and Cremer 1998; Branch 2001).

Numerous age estimation studies have been performed on orange roughy, the majority on populations from New Zealand and Australia. These studies have provided estimated age at maturity of between 23 and 40 years, a maximum age exceeding 100 years, and some estimates of ages exceeding 150 years (Smith et al. 1995; Tracey and Horn 1999; Green et al. 2002). Age estimation studies of populations in the North Atlantic and off Chile and Namibia have been guided by the New Zealand and Australian protocol and similarly concluded that orange roughy have centenarian life spans (Clark et al. 1999; Allain and Lorance 2000; Payá et al. 2006). However, there are alternative hypotheses based on an alternative interpretation of growth zones and modeling studies of otolith growth arguing that orange roughy are a relatively rapid-growing, short-lived species (e.g., Romanek and Gauldie 1996; Gauldie and Sharp 2001).

The most recent estimations of orange roughy age have utilized growth-zone counts from longitudinal thin sections of sagittal otoliths and made the assumption that the zone counts represent age (Tracey and Horn 1999). The resolution of growth zones in older otoliths has proven difficult, and the zone counts can have high uncertainty. In addition, a between-institute bias in zone count interpretation was documented (Francis 2006). Although the general consensus in the international community has been that orange roughy are a slow-growing, long-lived species, the argument that they are actually short-lived and fast-growing, combined with limited age validation studies to date and the imprecision and potential bias in otolith zone counts, has highlighted the need for further and conclusive age validation.

The age validation techniques that have been applied to deepwater fishes range widely in efficacy and precision (Campana 2001). Some techniques rely on establishing a temporal context to early growth by measuring changes in otolith zones or fish length; for instance, marginal increment analysis and length frequency analysis have been applied to orange roughy (Mace et al. 1990). The disadvantage of these techniques is that it is necessary to extrapolate the findings to provide support for older ages because of a loss of growth-zone or length-mode resolution. Other age validation methods rely on marking and recapturing older fish (e.g., oxytetracycline injection and tagging), but recovery of deepwater fishes is time-consuming and the survival rates are likely to be low (Campana 2001). Advances in the use of radioactive proxies for age have provided opportunities for independent age determination of deepwater fishes, and the primary techniques currently in use are bomb radiocarbon ($\Delta^{14}C$) and lead–radium dating, both of which can work well either independently or together (e.g., Andrews et al. 2007). However, for orange roughy, bomb radiocarbon dating is unsuitable because the species lacks an epipelagic phase in its life cycle, a requirement if the timing of the influx of bomb radiocarbon is to provide an accurate temporal marker (Kalish 2002).

Lead–radium dating relies on the incorporation of radium-226 (^{226}Ra), a naturally occurring calcium analog, from the environment into the otolith and its subsequent decay to lead-210 (^{210}Pb). By measuring the disequilibria of these two radioisotopes in otolith material, an independent estimate of age can be determined based on the known ingrowth rate of ^{210}Pb from ^{226}Ra (Smith et al. 1991). Fenton et al. (1991) applied lead–radium dating to orange roughy otoliths and provided radiometric age estimates that were the first to support a centenarian life span. In this study, it was necessary to pool a large number of whole otoliths to acquire enough material for measurement of ^{226}Ra, because the technology used at the time was not sensitive enough to detect ^{226}Ra at the low levels typically present in otolith material. It was also necessary to assume that ^{226}Ra was incorporated in constant proportion to otolith mass growth. In

addition, when considering the decay of ^{226}Ra to ^{210}Pb with respect to otolith growth, a gradient of ^{210}Pb activity is formed where core material has the highest ^{210}Pb:^{226}Ra activity ratio (the oldest part) decreasing to the outer layer (the youngest part). Because of the gradient, it was necessary to make an additional assumption: mass growth must be modeled by assuming that growth rates are known to some degree, which introduces circularity with respect to radiometric age determination. Francis (1995) described two approaches to avoid this assumption, reanalyzed the Fenton et al. (1991) lead–radium data, and reached a similar conclusion: a centenarian longevity was likely for orange roughy.

Increased sensitivity in the techniques used to determine ^{226}Ra activity has made it possible to reduce the sample size necessary to detect ^{226}Ra in otolith material with increased precision and accuracy. As a result, the first few years of growth can now be extracted from the core of orange roughy otoliths and analyzed for both ^{210}Pb and ^{226}Ra; hence, there is no need for sample weight dependence in calculating age or the assumption that ^{226}Ra uptake was constant (Andrews et al. 1999*a*; Campana 2001). In addition, advances in mass spectrometry have significantly reduced the error and processing time associated with the measurement of ^{226}Ra, which makes the measurement of ^{210}Pb the limiting factor in lead–radium dating (Andrews et al. 1999*b*). By using the same small sample of core material (the first few years of growth) for the measurement of both ^{210}Pb and ^{226}Ra activities, the problems associated with mass growth assumptions or variable uptake of ^{226}Ra are largely circumvented (Campana et al. 1990; Kimura and Kastelle 1995).

The goals of this study were (*i*) to provide independent age validation of orange roughy using the improved lead–radium dating procedure on orange roughy otolith cores and (*ii*) to address long-standing concerns over the validity of lead–radium dating as a tool for age determination in fishes. In addition, these findings provided an opportunity to discuss orange roughy age estimation and the importance of valid age determinations in light of recent research advances and perspectives.

Materials and methods

Samples of orange roughy otoliths used in this study were from archival collections previously aged using the growth-zone count method. The first otolith from each fish had been longitudinally thin-sectioned to allow growth-zone counting and the second "sister" otolith was used for the radiometric analyses. Otoliths were selected to form estimated age groups, which were chosen to create as narrow an age range for each age group, while covering as much of the life span as possible. In a feasibility study (Andrews and Tracey 2003), only samples aged by National Institute for Water and Atmospheric Research (NIWA) were used, but to obtain sufficient samples for older age groups, otoliths collected by the New Zealand Seafood Industry Council (SeaFic) and aged by Central Ageing Facility (CAF) in Australia were also used in the complete study. The number of otoliths required for orange roughy age groups was based on lead–radium information determined from the feasibility study. The specifics of sample composition (i.e., collection details of each fish used) were tabulated elsewhere (Andrews 2009).

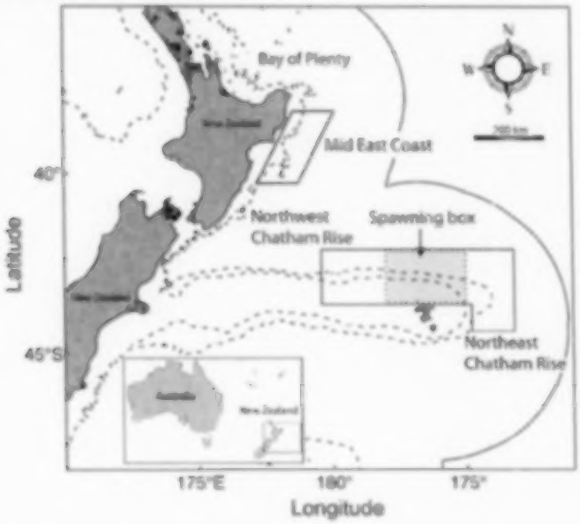
The orange roughy otoliths were selected from various regions off the north island of New Zealand (Fig. 1). For organizational purposes, the feasibility study remains separated from the follow-up age groups because of minor differences in protocol and development of the procedure used for the other groups. The youngest group analyzed was part of the feasibility study and consisted of whole juvenile otoliths collected from the Northwest Chatham Rise region in 1989 (group 0–2 years). The otoliths used in the adult samples were collected from the Chatham Rise and Bay of Plenty regions in 1984 (group 34–38 years), 1990 (group 61–71 years), and 1996 (group 70–81 years). In the following study, the samples analyzed primarily filled in the older age groups. These otoliths came from four regions (Spawning Box, Northeast and Northwest Chatham Rise, and Mid East Coast) and were all collected in 2002 and 2003. The age groups were as follows: ORH 1, 25–30 years; ORH 2, 40–45 years; ORH 3, 60–69 years; ORH 4, 70–80 years; ORH 5, 81–88 years; ORH 6, 90–108 years. Samples from the Spawning Box contributed exclusively to ORH 1 and ORH 2 and composed part of ORH 3 with Mid East Coast samples. The oldest age groups (ORH 4, ORH 5, and ORH 6) consisted of otolith samples from all regions. Note that each sample was a collection of aged otoliths that was processed once; no replication was possible in this study because of low sample availability and the limitations of sample size in the application of lead–radium dating (for a detailed discussion on lead–radium dating, see Andrews 2009).

Sample preparation and processing

Based on the dimensions and weight of 4-year-old whole otoliths from several samples made available for the feasibility study and from information on juvenile otolith weight data available from Mace et al. (1990), a target core sample size of 5.5 mm long × 2.5 mm wide × 0.8 mm thick, weighing approximately 0.012 g, was chosen. The proximal surfaces of each otolith were ground down by hand on a Buehler Ecomet III lapping wheel with 120- to 320-grit wet–dry silicon–carbide paper until the otolith was the target dimensions and weight. The resulting core looked very similar to a juvenile otolith in shape (pyramidal) and size. An exception was the group 0–2 years, which was processed whole. Hand-grinding was chosen for this study over the use of a micromilling machine primarily to follow the method used in the feasibility study and also because of the ease of this method.

It was ascertained from the feasibility study that dimensions alone were not enough to be certain that the core was not heavier than targeted size, as the average weight of cored otoliths was often found to be greater than expected (~0.024 g). To correct for this in this study, each core was first ground down using otolith dimensions as a guide, then weighed, and reground if the weight was greater than targeted. This was repeated until the target dimensions were achieved; in these cases, a weight of just under 0.02 g was targeted to avoid the loss of too much material and to allow for mass loss during the cleaning process (typically 3%–5%). Samples were cleaned of any adhering contamination following Andrews et al. (1999*b*). The weight of all of the cores and pooled otolith age groups were measured to the nearest 0.1 mg.

Fig. 1. Areas within the New Zealand exclusive economic zone (EEZ) referred to in the text from which orange roughy (*Hoplostethus atlanticus*) otolith samples were collected and used in the lead–radium analyses.



Radiochemical protocol

A detailed protocol describing sample preparation, chromatographic separation of ^{228}Ra from barium and calcium, and analysis of ^{228}Ra using mass spectrometry was described by Andrews et al. (1999*b*). These procedures have not changed for this study, except for two aspects of the analysis: (*i*) radium recovery was improved by shifting the collection interval on the final chromatography column to begin after the first 200 μL (as opposed to after 250 μL), and (*ii*) purified radium samples were analyzed using an improved inductively coupled plasma mass spectrometry (ICPMS) technique, which is less prone to ionization suppression (Craig Lundstrom, University of Illinois–Urbana Champaign, Department of Geology, Urbana, Illinois, USA, unpublished data).

^{228}Ra analyses were performed by isotope dilution techniques following the procedure of Andrews et al. (1999*b*), except that analyses in this study were performed using a Multicollector ICPMS in place of TIMS. The new mass spectrometer analysis took place on a Nu Plasma™ HR instrument located in the Department of Geology at University of Illinois–Urbana, Champagne. The analysis introduced the chemically purified radium into a desolvating nebulizer as a 2% HNO_3 solution. The sample was converted into dry aerosols, which were swept into the argon plasma and the mass spectrometer. Sensitivity during radium analysis corresponds to approximately 25 counts per second (cps)·ppq^{-1} radium at an uptake rate of 70 μL·min^{-1}. Once in the spectrometer, isotope ratios were measured by switching the magnet back and forth between atomic mass units (amu) 226 and 228 after a 1-min background taken at amu 225.5 or 227.5. An individual sample analysis consisted of 10 measured ratios representing 10 s integrations on each amu peak. After each analysis, the system was washed successively with 5% HNO_3 and 2% HNO_3 for 10 min until no residual radium signal could be observed. Count rates on samples in this study were usually >100 cycles·s^{-1} for each radium isotope, compared with the nominal background on clean acid, which was <1 cycle·s^{-1}.

Radiometric age was determined from the measured ^{210}Pb and ^{226}Ra activities. Because the activities were measured using the same sample, the calculation was independent of sample mass. Radiometric age was calculated using an equation derived from Smith et al. (1991) to compensate for the ingrowth gradient of ^{210}Pb–^{226}Ra in the otolith core:

$$t_{age} = \left[\ln\left(\frac{1 - \left(\frac{A^{210}Pb}{A^{226}Ra}\right)}{(1 - R_0)\left(\frac{1-e^{-\lambda T}}{\lambda T}\right)} \right) / (-\lambda) \right] + T$$

where t_{age} was the radiometric age at the time of analysis, A^{210}Pb was the measured ^{210}Pb activity at time of analysis (reported as disintegrations per minute per gram (dpm·g^{-1})), A^{226}Ra was the ^{226}Ra activity measured using ICPMS (reported as dpm·g^{-1}), R_0 was the activity ratio of ^{210}Pb and ^{226}Ra initially incorporated, λ was the decay constant for ^{210}Pb (ln(2)·22.26 year^{-1}), and T was the estimated core age based on the first few growth zones. An initial uptake ratio of R_0 = 0.0 was used based on the close agreement of the measured juvenile age group lead–radium ratio with the expected ingrowth curve; however, other studies have accounted for what appeared to be exogenous ^{210}Pb with minor adjustments necessary (e.g., Kastelle et al. 2000; Stransky et al. 2005). A radiometric age range, based on the analytical uncertainty, was calculated for each sample by using error propagation through to the final age determinations (2 standard errors, SE). Calculated error included the standard sources of error (i.e., pipetting, spike, and calibration uncertainties, etc.), alpha-counting statistics for ^{210}Pb, and the ICPMS analysis routine.

The 95% confidence interval (2 SE) from lead–radium dating was used to interpret the validity of growth-zone counts and to determine the strength and limits of age confirmations. To describe the trend between age estimation from growth-zone counts and lead–radium dating, a simple linear regression was applied. Age agreement or disagreement between the methods, in terms of potential ageing bias, were given considering the potential error (2 SE).

Results

The six age groups ORH 1 to ORH 6 covered a range of prerecruitment juveniles to old adults and consisted of groups of 13 to 32 individual otolith cores, with an age group sample weight of 0.227–0.464 g (Table 1). The mean core weight was consistent and close to the target weight of a 4-year-old otolith (~0.012 g). The results from three of the four feasibility study age groups were added to provide more lead–radium dating results, but a sample that experienced accidental loss of ^{228}Ra tracer during the spike addition process (group 61–71 years) was not included (Andrews and Tracey 2003). Sample weight for the feasibility study ranged from a very low weight for the juvenile sample (0.040 g) to the highest group weight approaching 1 g (0.842 g). Mean core weights for the feasibility study were slightly higher because they were extracted in the exploratory phase of the overall study.

Table 1. Summary of the orange roughy (*Hoplostethus atlanticus*) samples processed in this study and in the previous feasibility study.

	Zone count group (years)	Mean zone count (years)	Time since capture (years)	No. of otoliths	Sample weight (g)	Mean core weight (g)
ORH sample series						
ORH 1	25–30	28	2.7	31	0.426	0.0137
ORH 2	40–45	42	2.7	32	0.464	0.0145
ORH 3	60–69	65	3.0	24	0.402	0.0168
ORH 4	70–80	74	3.1	26	0.339	0.0130
ORH 5	81–88	85	2.9	14	0.242	0.0173
ORH 6	90–108	98	2.7	13	0.227	0.0175
Feasibility study	0–2	2	13.3	9	0.040	0.0044
	34–38	36	18.7	37	0.842	0.0228
	61–71[a]	66	12.7	20	0.474	0.0237
	70–81	76	7.0	28	0.701	0.0250

Note: Mean estimated growth-zone count and average time since capture for each group, with resultant pooled sample number and weight, are given. An average core weight for each sample is given as an indicator of the consistency of coring.

[a]This sample was not considered further in this study because of the accidental loss of the radium-228 tracer during the spike addition process (Andrews and Tracey 2003).

Table 2. Radiometric results for orange roughy (*Hoplostethus atlanticus*).

	Zone count group (years)	^{210}Pb activity	^{226}Ra activity	^{210}Pb:^{226}Ra activity ratio	2 SE
ORH sample series					
ORH 1	25–30	0.0426±6.1	0.0784±2.20	0.5437	0.0350
ORH 2	40–45	0.0591±4.9	0.0755±2.17	0.7824	0.0418
ORH 3	60–69	0.0696±5.3	0.0795±2.18	0.8761	0.0498
ORH 4	70–80	0.0759±5.2	0.0826±2.24	0.9189	0.0524
ORH 5	81–88	0.0624±9.3	0.0672±3.19	0.9285	0.0915
ORH 6	90–108	0.0693±5.5	0.0688±2.56	1.0067	0.0606
Feasibility study[a]	0–2	0.0647±18	0.2090±5.43	0.3096	0.0583
	34–38	0.0522±4.4	0.0692±2.99	0.7549	0.0399
	70–81	0.0561±4.6	0.0599±5.14	0.9365	0.0644

Note: Listed are the estimated growth-zone count ranges and measured ^{210}Pb and ^{226}Ra activities for all samples ± 2 standard errors (SE) for the measured activities. Sample activities for ^{210}Pb and ^{226}Ra were reported in disintegrations per minute per gram (dpm·g^{-1} ± percent error).

[a]One sample was eliminated from the original four age groups because of the accidental loss of the radium-228 tracer during the spike addition process (Andrews and Tracey 2003).

The activities of ^{210}Pb and ^{226}Ra were measured for all samples (Table 2). The activity of ^{210}Pb was relatively inconsistent with increasing age, as would be expected for samples with consistent ^{226}Ra levels; however, the variable activity of ^{226}Ra compensated for what would have been a random age distribution, the result being a steady increase in the ^{210}Pb:^{226}Ra activity ratio with increasing estimated age. It is interesting to note that the measurable activities from the extraordinarily small juvenile sample were possible because the activity of ^{226}Ra was relatively high and the overall age (time since collection plus average age) was 14 years. The range of sample contribution of ^{226}Ra to the measured radium ratios using ICPMS was 9%–72%, which accounts for some of the variability in measurement error.

The estimated age of the samples from the growth-zone counts were similar to the radiometric ages determined from the measured ^{210}Pb:^{226}Ra activity ratios (Table 3). There was considerable agreement for the sample series, with one age group not in agreement (feasibility study age group 34–38 years) based on the margin of error from lead–radium dating (2 SE). This sample was aged lower using lead–radium dating than the estimate from growth-zone counts.

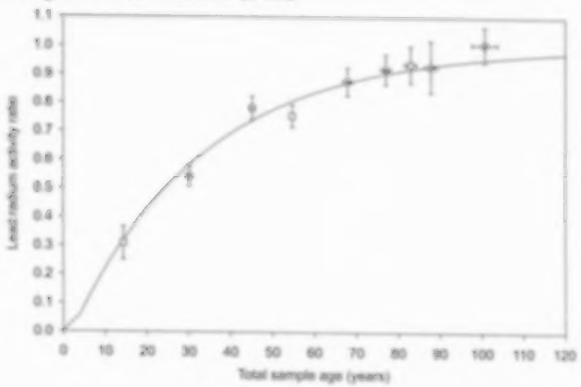
Correspondence of the measured ^{210}Pb:^{226}Ra ratios with the expected ingrowth curve indicated that growth zone derived age estimates were precise (Table 3), within a level of uncertainty that increases with age, and provides support for orange roughy age estimation procedures (Fig. 2). This plot is only possible for total sample age (age from growth-zone counts plus the time since capture). For all but two of the samples, the measured ratio fell within 2 SE of the expected ingrowth curve. The youngest sample (total sample age of about 14 years) was in close agreement with the expected ratio, indicating that the effect of exogenous ^{210}Pb was low or negligible. This was the basis for choosing an initial uptake ratio (R_0) equal to zero.

Table 3. Summary of growth-zone counts and radiometric ages for orange roughy (*Hoplostethus atlanticus*).

	Zone count group (years)	Mean zone count (years)	Radiometric age (years)	Radiometric age range (years)
ORH sample series				
ORH 1	25–30	28 (±10)	24	22–27
ORH 2	40–45	42 (±15)	48	42–55
ORH 3	60–69	65 (±23)	66	55–82
ORH 4	70–80	74 (±26)	79	63–113
ORH 5	81–88	85 (±30)	83	57–undefined
ORH 6	90–108	98 (±35)	Undefined	93–undefined
Feasibility study	0–2	2 (±2)	1	0–3
	34–38	36 (±13)	29	24–34
	70–81	76 (±27)	83	61–undefined

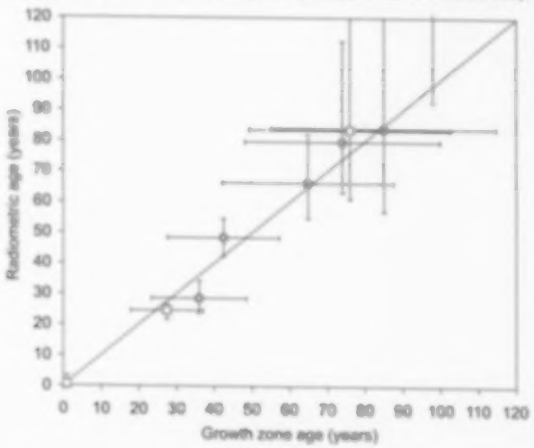
Note: Mean growth-zone age and radiometric age were calculated from the measured ^{210}Pb:^{226}Ra activity ratios and corrected for time since capture. Radiometric age range is 2 standard errors (SE) from the analytical uncertainty and error propagation. A potential uncertainty for otolith zone counts using 2 SE from the age-misclassification matrix, used in New Zealand stock assessments, is provided to illustrate potential age determinations.

Fig. 2. Plot of the measured ^{210}Pb:^{226}Ra ratios with respect to total sample age (mean growth-zone count plus the time since capture) for orange roughy (*Hoplostethus atlanticus*) samples processed in this study (shaded circles) and in the feasibility study (open squares), plotted with the expected ^{210}Pb:^{226}Ra ingrowth curve. Horizontal error bars (some within the symbol size) represent 2 standard errors (SE) around the mean of the sample growth-zone age determined by National Institute of Water and Atmospheric Research (NIWA) and Central Ageing Facility (CAF). The vertical error bars represent the analytical uncertainty associated with measuring the ^{210}Pb:^{226}Ra ratio (2 SE).



An age-agreement plot allowed for direct comparison between the mean growth-zone count and the radiometric age (Fig. 3). One data point (ORH 6) could not be plotted as it had a measured ratio that narrowly exceeded 1.0 (Table 2), resulting in an undefined radiometric age (Table 3). Correlation between the two ageing methods was good (R^2 = 0.979). The slope of the regression was close to 1.0 (regression slope = 1.08), indicating that there was general agreement between the methods; however, this result does not take into consideration the change in radiometric age uncertainty as the measured ^{210}Pb:^{226}Ra activity ratio increases. An estimate of zone-count error from other studies was included in Fig. 3 out of interest and provided an indication of a potentially wide margin of uncertainty (Dunn 2006; A. Hicks, School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat Street, Seattle, WA 98105, USA, unpublished data). By treating the radiometric age determinations as independent estimates of age, using core material, and making no assumptions with regard to

Fig. 3. Orange roughy (*Hoplostethus atlanticus*) age agreement plot of growth-zone age estimates versus radiometric age estimates (corrected to time of capture) for samples processed in this study (shaded circles) and in the feasibility study (open squares), plotted with a line of agreement for comparison. Out of interest, the horizontal error bars indicate the potential uncertainty in otolith zone counts (2 standard errors, SE), derived from an age-misclassification matrix used in New Zealand stock assessments (Dunn 2006; A. Hicks, University of Washington, School of Aquatic and Fishery Sciences, Seattle, WA 98105, USA, unpublished data). Vertical error bars represent the analytical uncertainty associated with measuring ^{210}Pb and ^{226}Ra (2 SE). The upper end of some error bars and the age of one sample exceed the figure limits because age (or its upper bound) was undefined (the ^{210}Pb:^{226}Ra ratio exceeded 1.0).



otolith growth, the results indicated that otoliths in the oldest age group were at least 93 years old.

Discussion

The application of lead–radium dating to orange roughy otolith cores was successful, with good agreement between the mean age from growth-zone counts and the lead–radium technique. Use of otolith core material avoided potential problems associated with the application of otolith mass growth models and provided the most accurate age determinations to date for orange roughy. The minor assumption typically associated with the use of a known-age core was well supported by the age validation study performed by Mace et al. (1990) on otolith margin type, where the first few years of growth were well documented. The radiometric findings provided refined support for age estimates that exceed 100 years, even though the uncertainty in radiometric age increased as lead–radium ratios approached one. Independent of other age estimation procedures, lead–radium dating in this study provided support for a centenarian life span.

Making precise and accurate growth-zone counts appears to be difficult for orange roughy. Francis (2006) described calibration data sets used in New Zealand and Australia in which the same otoliths were read twice, some involving replicate readings by the same reader and some involving different readers from the same or different institutions. The variability in growth-zone counts was found to be least when a single reader re-read the otoliths, then greater for two readers from the same institute, and greater again for two readers from different institutions. There was also substantial variability in what each reader considered to be a readable otolith. An age-misclassification (error) matrix was constructed from these data for use in stock assessment models and was estimated from the between-institute comparisons, as it was believed that this would introduce a larger and more appropriate variation for the assigned age range around the true range (Dunn 2006; A. Hicks, School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat Street, Seattle, WA 98105, USA, unpublished data). The imprecision in ageing estimated from this analysis was substantial; for example, the 95% confidence interval for an orange roughy aged at 40 years was estimated to be 26–54 years.

Perhaps of more concern has been a suspected bias in age estimation. This suspected bias resulted in age-frequency data being excluded from use in stock assessments in New Zealand, the rationale being that there was insufficient confidence that differences observed between samples were real and not simply artifacts arising from ageing problems. One possible source of bias appears to be associated with the detection of the transition zone (TZ), a region on the otolith where the width of the growth zone changes from wide to narrow, assumed to be the result of a switch from primarily somatic growth to gamete production at the onset of maturity (Francis and Horn 1997). Francis (2006) found that there was a tendency to count more growth zones, therefore to age older, when a TZ was recorded, with a relative bias of about 6 years. This would result in an ageing problem around the ages when the TZ was formed, roughly between 20 and 45 years, and could explain the offset in the age group 34–38 years.

Francis (2006) also found that estimated age at length varied substantially between years and areas in a way that could not be attributed easily to real changes in population structure. For example, estimated mean age at length in one specific area changed by 30% over a 2-year period. If orange roughy were growing slowly, then it should not be possible for age at length to change so much over a short period: this observation is therefore likely to be either a sampling or an ageing bias. Francis (2006) then demonstrated likely ageing biases occurring in the calibration data sets; the estimated biases were greatest between institutes, at about 10%, but varied in direction depending on the otolith sample considered. For the present study, imprecision in growth-zone counts adds uncertainty to the age composition of the age groups but does not change the conclusions about overall longevity.

For orange roughy, examination of otolith margin growth and length frequencies provided the first temporal evidence for a life span greater than 50 years, but this estimate was based on trends observed with small fish extrapolated to the largest fish (Mace et al. 1990). Further support for relatively high longevity came from one of the earliest applications of lead–radium dating to fishes (Fenton et al. 1991; Francis 1995) and shortly thereafter with an application of radiocarbon dating (Kalish 2002). Although radiocarbon dating (not to be confused with bomb radiocarbon dating) provided support for a centenarian life span, with rough age estimates that were complicated by other factors, lead–radium dating was the first to begin to provide more concrete evidence.

The seminal radiometric study on orange roughy by Fenton et al. (1991) used pooled whole otolith samples and an approach that made it necessary to make some educated assumptions. The study did not perform any growth zone derived age estimations, but instead sought to independently determine age for groups of fish with similar length and otolith weight. For the method to work, there needed to be little or no (*i*) between-individual variability in otolith mass growth rate and (*ii*) variability in ^{226}Ra uptake throughout the growth of the otolith. In addition to these factors, there were important assumptions about the very nature of the lead–radium dating technique; the otolith needed to be a closed system to (*i*) the ingrowth of ^{210}Pb from ^{226}Ra with no loss of ^{226}Ra daughter products and (*ii*) the incorporation of exogenous sources of the decay series radionuclides (not from the decay of incorporated ^{226}Ra). Within these assumptions, the most conceivable problems were (*i*) the potential loss of ^{222}Ra (daughter product of ^{226}Ra and a noble gas) and (*ii*) the incorporation of exogenous ^{210}Pb or polonium-210 (used as a proxy for ^{210}Pb in alpha-spectrometry). These concerns could not be thoroughly addressed when Fenton et al. (1991) performed the analyses on orange roughy otoliths, but subsequent studies have provided support for the assumptions that were necessary (Francis 1995; Smith et al. 1995). In some cases, the assumptions have been tested directly.

The veracity of the lead–radium technique was questioned by a number of papers addressing the assumptions. In a perspective paper, West and Gauldie (1994) argued that there were uncontrollable errors that invalidated the technique for

fish age estimation but provided a series of observations and conclusions that failed to consider equally viable explanations that do not discredit the radiometric technique. In an attempt to measure loss of ^{222}Ra from orange roughy otoliths, Gauldie and Cremer (1998) openly recognized anomalous results, a faulted technique, and negative values that imply the contrary; nevertheless, they concluded from weak trends in rather dubious data that loss of ^{222}Ra was a problem and that this invalidated old ages. The inconclusive quality of those data was reiterated in a follow-up application that reported a loss of 26%–28% (Gauldie and Cremer 2000), but these findings are inconsistent with determinations made in this study because large losses as such would create an upper limit to the measured lead–radium ratios. Furthermore, evidence supporting the author's conclusions was cited (e.g., incomplete concepts from a report by Whitehead and Ditchburn (1996)), but a prior publication that (*i*) specifically addressed ^{222}Ra diffusion from orange roughy otoliths and (*ii*) provided evidence to support a long life span (Whitehead and Ditchburn 1995) was ignored (i.e., Gauldie and Cremer 1998, 2000; Gauldie and Romanek 1998).

Perhaps the most scientifically robust study to date on potential loss of ^{222}Ra from otoliths was performed on red snapper (*Lutjanus campechanus*) and red drum (*Sciaenops ocellatus*) from the Gulf of Mexico (Baker et al. 2001). In this study, two species with relatively large otoliths and some of the highest ^{226}Ra activities recorded from otolith material were analyzed; the study resulted in low (≤4.1%) to no loss of ^{222}Ra. The measured loss was further considered more of a surface emanation because larger otoliths liberated relatively less ^{222}Ra. They concluded that such losses were probably insignificant relative to other sources of error associated with lead–radium dating. This would be especially true in the case of otolith cores because the loss would be temporary and much less significant with time. In another study, Pacific halibut (*Hippoglossus stenolepis*) otoliths revealed no evidence of ^{222}Ra loss, but experimental error from low lead–radium levels led to somewhat inconclusive results (Kastelle and Forsberg 2002).

In the present study, a small group of juvenile otoliths provided indirect evidence, similar to the approach used by Kastelle and Forsberg (2002), that ^{222}Ra loss was not a significant factor for orange roughy. These whole otoliths were aged at 0 to 2 years but were collected 13.3 years prior to analysis for lead–radium content. The measured ratio was consistent with the total sample age of approximately 14 years with a margin of error of about 2 years. If the loss of ^{222}Ra for this sample was on the order of 25%, as was proposed by Gauldie and Cremer (2000), then radiometric age would have been approximately 6 years less than the known age of 14 years of the sample itself. The scenario was similar for other studies in which juvenile otoliths have been used to determine early ingrowth ratios (e.g., Andrews et al. 2005, 2007).

Another scenario to consider with regard to loss of ^{222}Ra is the potential affect on the measured lead–radium ratio as age approaches 100 years. In a study performed on yelloweye rockfish (*Sebastes ruberrimus*), otolith cores provided measured lead–radium ratios that were very close to secular equilibrium (Andrews et al. 2002). If there was a consistent problem with the loss of ^{222}Ra throughout the period of otolith growth, then secular equilibrium would not and could not be attained. It was at approximately 99 years that secular equilibrium was approached to within 95% for the ingrowth model used in the present study (core age of 4 years). Provided that there was a ^{222}Ra loss on the order of 25% for the oldest orange roughy age group (90–108 years with an average age of 98 years), the measured ratio (1.007, 2 SE low of 0.9461) would have been far less, approximately 0.675 and a radiometric age of 30–40 years. In addition, none of the samples analyzed in this study, as well as other studies (e.g., Andrews et al. 2002), would have exceeded this ratio. The findings presented here for orange roughy support the hypothesis that loss of ^{222}Ra was not a significant factor in the determination of age from lead–radium dating.

The potential problem of ^{226}Ra uptake variability was largely circumvented with the use of otolith cores (Campana et al. 1990), but detection limits for ^{226}Ra combined with the small otolith core size for orange roughy precluded an analysis of otolith core material by Fenton et al. (1991). Although it is possible that otoliths with consistent ^{226}Ra levels were measured in the whole otolith study, more support was necessary to alleviate concerns about this assumption. Based on a comprehensive synopsis, ^{226}Ra uptake can vary considerably for orange roughy, with a range of approximately 0.05–0.2 dpm·g^{-1} (Andrews 2009); however, the present study provided additional support that ^{226}Ra activity levels can also be relatively consistent among the core samples (average activity of ^{226}Ra = 0.0740 ± 0.0053 dpm·g^{-1}, n = 9). The notable exception was the whole juvenile age group (0.209 ± 0.011 dpm·g^{-1}). This group consisted of otoliths that were very small, with an average weight that was about three to five times lower than the average core weight. This finding implies that within the first 2 years of growth, ^{226}Ra uptake may be greater relative to later in life. The reason for this finding is unknown but may be attributed to differences in habitat, diet, metabolism, or all of the above. Relatively high variability of ^{226}Ra activities measured in other studies reinforce the hypothesis that uptake can be highly variable and that mass growth rate models using an average ^{226}Ra activity provide radiometric ages that should have greater uncertainty than was assumed at the time. Although the radiometric ages in this study support those of Fenton et al. (1991), this study also places emphasis on the necessity for sample-specific lead–radium measurements to avoid ^{226}Ra uptake assumptions (Francis 2003).

In an age and growth study of Northeast Atlantic orange roughy, lead–radium dating was applied to whole otoliths following a technique similar to that of Fenton et al. (1991), but the results were considered somewhat inconclusive (Allain and Lorance 2000). In the study, age estimates were made by counting growth zones in whole and thin-sectioned otoliths and indicated a high degree of age-estimate departure between whole otolith and thin-section ageing beginning at approximately 40 years. The finding was similar to the classical plot discovered for Pacific ocean perch (*Sebastes alutus*; Beamish 1979), indicating that there were differences in age on the order of decades between whole otolith and thin-section ageing. Allain and Lorance (2000) did not consider the lead–radium results as conclusive be-

cause of ageing imprecision and what appeared to be inconclusive lead–radium results (P. Lorance, IFREMER, 150 quai Gambetta, BP 699, 62321 Boulogne-sur-mer, France, personal communication). Use of successive dissolutions on one otolith by Allain and Lorance (2000) provided similar results to the findings of Whitehead and Ditchburn (1996). Both dissolution studies were performed making the assumption that the core material (youngest) was at the center of the otolith and that successive acid dissolutions would provide greater and greater ^{210}Pb activities; however, both studies revealed anomalous distributions of ^{210}Pb and concluded that the growth of the otolith was not concentric (Whitehead and Ditchburn 1996; P. Lorance, personal communication). The findings in Whitehead and Ditchburn (1996) have been cited as support for the argument that lead–radium dating cannot be applied to otolith material (Gauldie and Cremer 1998, 2000), yet the observable reason for the anomalous results was invalid assumptions about otolith growth geometry.

Allain and Lorance (2000) cite that the mean ages for the four otolith groups analyzed ranged from 29 to 80 years. An analysis of the lead–radium data (provided by P. Lorance, personal communication) and the margin of error associated with the measurements of ^{210}Pb and ^{226}Ra revealed that the minimum age for fish in the samples was 45 years (2 SE). This determination was conservative because the measured ratios were from whole otolith samples and age would have been diluted by younger material. These findings provided support for older age estimates obtained from thin sections in that study and provided additional support for hypotheses that challenge low longevity estimates for orange roughy.

Although there have been challenges to the centenarian longevity assertion for orange roughy, this assumption has been accepted for some time in stock assessment and fishery management. The alternative hypothesis of low longevity and rapid growth would be associated with greater productivity, but the repeated failure of orange roughy stocks to rebuild rapidly following catch reductions gives further evidence that this hypothesis is not correct. Moreover, recent stock assessments and analyses have suggested productivity since the inception of the fisheries may be even lower than is currently being assumed (Sissenwine and Mace 2007; M.R. Dunn, unpublished data). Fishing down the age structure may have additional impacts on sustainability that have yet to be fully considered (Berkeley et al. 2004; Beamish et al. 2006; Cailliet and Andrews 2008). For a long-lived species like orange roughy, relatively low fecundity and highly episodic recruitment also contribute to their low productivity (Pankhurst and Conroy 1987; Longhurst 2002).

Based on reasonable assumptions that were supported by orange roughy growth data (Smith et al. 1995), the radiometric data presented by Fenton et al. (1991) supported a maximum age for orange roughy that exceeded 84 years (Francis 1995). The findings of the present study provide even more robust support for the centenarian age estimates obtained from otolith thin sections. As independent estimates of age, the radiometric results indicated that fish in the oldest age group were at least 93 years old, and by circumventing assumptions that were previously necessary, lead–radium dating of otolith core material has validated a centenarian life span for orange roughy.

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Recent sedimentary legacy of sockeye salmon (*Oncorhynchus nerka*) and climate change in an ultraoligotrophic, glacially turbid British Columbia nursery lake

Aaron C. Hill, Jack A. Stanford, and Peter R. Leavitt

Abstract: Paleolimnological studies of sockeye salmon (*Oncorhynchus nerka*) nursery lakes have shown that lake trophic status is often regulated by climate and harvest via marine-derived nutrients (MDN) from adult spawners. However, these controls are not well understood for sockeye nursery lakes in coastal British Columbia, many of which are ultraoligotrophic and glacially turbid. We examined climate, sockeye population dynamics, and sedimentary indicators of lake algal production from 1958 to 2005 using a radioisotope-dated sediment core from Kitlope Lake, British Columbia. Despite high sedimentation rates (~4.7 mm·year^{-1}), significant influence of terrestrial and aquatic organic matter from the main tributary, and the lowest mean (± standard deviation) $\delta^{15}N$ (–0.28‰ ± 0.79‰; a proxy for MDN flux) yet reported from a sockeye nursery lake, sedimentary $\delta^{15}N$, C–N ratio, and fossil pigments were coherent with order-of-magnitude changes in sockeye escapements. Moreover, air temperatures were positively correlated with $\delta^{15}N$, indicating a climate influence counteracting declines of MDN import related to declining spawner returns. Despite elevated production potential, Kitlope Lake remains nutrient-limited with a declining sockeye population, and the productivity of this system would benefit from increased sockeye returns.

Résumé : Des études paléolimnologiques de lacs de nourricerie de saumons rouges (*Oncorhynchus nerka*) ont démontré que le statut trophique du lac est souvent contrôlé par le climat et les récoltes par l'intermédiaire des nutriments d'origine marine (« MDN ») apportés par les reproducteurs adultes. Cependant, ces contrôles restent mal compris dans les lacs de nourricerie de saumons rouges de la région côtière de la Colombie-Britannique, dont plusieurs sont caractérisés par une ultraoligotrophie et une turbidité d'origine glaciaire. Nous avons étudié de 1958 à 2005 le climat, la dynamique de population des saumons rouges et les indicateurs sédimentaires de la production lacustre d'algues dans une carotte de sédiments soumise à la datation par les radio-isotopes dans le lac Kitlope, Colombie-Britannique. Malgré de forts taux de sédimentation (~4,7 mm·an^{-1}), une influence notable de la matière organique terrestre et aquatique provenant du tributaire principal et la valeur moyenne (± écart type) de $\delta^{15}N$ (–0,28 ± 0,79 ‰; une mesure de remplacement du flux de MDN) la plus basse jamais observée dans un lac de nourricerie de saumons rouges, les valeurs de $\delta^{15}N$, de C:N et de pigments fossiles dans les sédiments sont compatibles avec les changements de l'ordre de 10 fois dans les échappements de saumons. De plus, il y a une corrélation positive entre les températures de l'air et $\delta^{15}N$, ce qui indique que l'influence du climat compense le déclin de l'apport de MDN dû à la diminution des retours de reproducteurs. Malgré son potentiel élevé de production, le lac Kitlope demeure limité par les nutriments avec une population déclinante de saumons rouges; la productivité du système bénéficierait d'une augmentation des retours de saumons rouges.

[Traduit par la Rédaction]

Introduction

Historic patterns in land air temperature, precipitation, streamflow, and biological productivity of marine and freshwater ecosystems are linked to climatic regime shifts in the North Pacific Basin (Mantua et al. 1997). At the continental scale, Pacific salmon (*Oncorhynchus* spp.) productivity has been remarkably coherent with these climate dynamics (Mantua et al. 1997; Beamish et al. 1999; Finney et al. 2000), although there is often high variance within individual fishing districts (Hilborn et al. 2003; Brock et al. 2007). Furthermore, the additive effects of climatic productivity constraints, overharvest, habitat loss, food web changes, and hatchery production have caused severe declines in many wild populations (Nehlsen et al. 1991; Lichatowich 1999).

Pacific salmon provide substantial quantities of marine-derived nutrients (MDN; nitrogen, phosphorus, and carbon) to terrestrial and freshwater food webs, including lakes,

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when adult fish return to natal freshwaters where they spawn and die (reviewed in Gende et al. 2002; Naiman et al. 2002). Sockeye salmon (*Oncorhynchus nerka*) juveniles rear extensively in lakes, where their growth rates are primarily regulated by food (zooplankton) availability and temperature (Edmundson and Mazumder 2001). Because sockeye obtain >95% of their mass as predators within marine food webs (Groot and Margolis 1991), anadromous salmon are highly enriched in $\delta^{15}N$ compared with other freshwater organisms. Thus, the density of sockeye spawners is positively correlated with $\delta^{15}N$ deposited in lake sediments (Finney et al. 2000; Brahney et al. 2006). Recent paleolimnological studies in Southwest Alaska sockeye nursery lakes, where spawner densities are typically high ($\gg 10^3$ fish·(km^2 lake area)$^{-1}$), have shown strong coherence among variations in climate and (or) salmon harvest and estimated salmon abundance ($\delta^{15}N$) during the past few centuries, further correlated to changes in past lake production as estimated from fossil diatoms and pigments (Finney et al. 2000; Gregory-Eaves et al. 2004; Schindler et al. 2005*a*). However, very few paleoecological studies have quantified the relative effect of climate and salmon abundance on nursery lake ecology in other important sockeye-producing regions, including coastal British Columbia (BC; Holtham et al. 2004; Brahney et al. 2006), where interactions between climate change and fertility are generally understudied (Melack et al. 1997; Schindler 2001; Tyedmers and Ward 2001).

The watersheds of BC's north and central coastal regions contain natal habitats for some 2500 identified salmon stocks, including many sockeye salmon populations that spawn and rear in cold, ultraoligotrophic lakes (Price et al. 2008). Many of these lakes are influenced by both coastal precipitation and large glaciers producing highly variable, turbid runoff and rapid lake-flushing rates, thereby reducing lake productivity (Shortreed et al. 2001) and presumably muting lake responses to MDN flux, as well as obscuring paleolimnological records of past lake change (Holtham et al. 2004). Despite cold thermal regimes and low plankton productivity, many glacially turbid BC sockeye nursery lakes historically had abundant returns of adults (e.g., Owikeno Lake, $>10^4$ fish·(km^2 lake area)$^{-1}$; Riddell 2004) and supported important commercial fisheries for over a century (Argue and Shepard 2005). Several sockeye stocks in this region have declined by 50% or more compared with historic run sizes (Riddell 2004; Price et al. 2008), possibly leading to a reduction in the fertility of natal lakes as MDN import was attenuated (Larkin and Slaney 1997; Schoonmaker et al. 2003; Stockner and Ashley 2003).

We used time-series paleolimnological analyses to evaluate the relative importance of climate and MDN from sockeye salmon as controls of the fertility of glacially turbid Kitlope Lake, a culturally important sockeye nursery lake in northern coastal BC. We correlated sedimentary proxies of lake production (total carbon, total nitrogen, fossil pigments), organic matter source ($\delta^{13}C$ and the C–N ratio), and MDN ($\delta^{15}N$) to estimated sockeye escapements and climate dynamics during the period 1958–2005. The average contribution of MDN to overall lake N influx was extremely low (<2%), and $\delta^{15}N$ was the lowest yet reported worldwide for a sockeye nursery lake. Nonetheless, lake fertility increased coincident with briefly elevated spawner densities, whereas the C–N ratio decreased, and total N, total C, $\delta^{13}C$, $\delta^{15}N$, and phytoplankton pigments all increased concomitant with a warming trend. Despite an apparent climate-induced increase in production potential, this sockeye nursery lake remains extremely nutrient-limited, and production and growth of juvenile sockeye may benefit from enhancement of marine nutrient import via increased escapement of spawners.

Materials and methods

Site characteristics

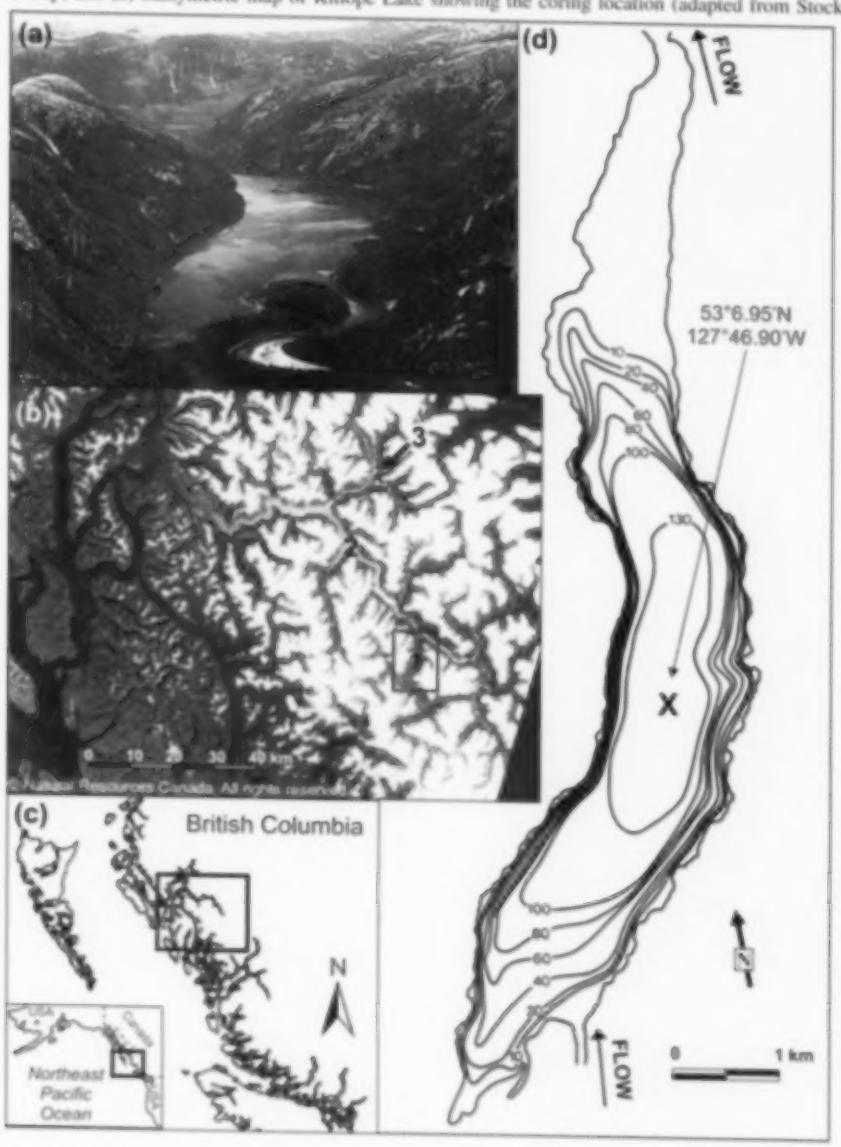
The Kitlope catchment drains into a long, narrow fjord (Gardner Canal) on the northern coast of British Columbia, Canada (53°15′N, 127°55′W; Fig. 1). At 2750 km^2, it is the largest roadless and unlogged coastal rainforest ecosystem in the temperate regions of the world (Travers 1991), allowing for paleoecological analysis in the absence of proximate anthropogenic disturbance. The catchment is part of the traditional territory of the Haisla First Nation, is protected as a provincial conservancy, and is considered a salmon sanctuary (Rahr and Augerot 2005). It includes two major subbasins, the Kitlope River and the Tezwa River, with Kitlope Lake (11.9 km^2) located at the lower end of the Tezwa River catchment (Fig. 1).

The climate in the Kitlope watershed is determined by wet, warm conditions related to ocean proximity in lower elevations interacting with colder continental air masses spilling into the headwaters. The local topography is steep, with several permanent glaciers in the headwaters region (Fig. 1*b*). Daily temperature and precipitation data for 1951–2006 were obtained from the Kemano weather station (Environment Canada 2007) 46 km north of Kitlope Lake (Fig. 1*b*). Mean annual temperatures and total precipitation were calculated from mean monthly values, and years with missing monthly values (1951, 1959, 1972, 1985, 1989–1990, and 1993–1994) were excluded or replaced with interpolated (5-point average) values. Pacific Decadal Oscillation (PDO) index values were obtained from the Web site of the Joint Institute for the Study of Atmosphere and Oceans (JISAO, University of Washington and National Atmospheric and Ocean Administration) (2007) for comparison with temperature data, sockeye escapement estimates (Spilsted and Spencer 2009), and sedimentary proxies of lake trophic status. Local and traditional ecological knowledge gathered through semidirected interviews (Huntington 2000) with Haisla elders provided additional perspective on local climate patterns.

In addition to the pristine nature of the catchment, we selected Kitlope Lake for this study based on its extreme oligotrophy and strong glacial influence. The lake is cold, weakly stratified, glacially turbid, mildly acidic, poorly buffered, and ice-covered most winters (Stockner et al. 1993). Plankton biomass is low and species-poor; chlorophyll concentration, primary production (Table 1), and juvenile sockeye size and density in Kitlope Lake are among the lowest measured for any large sockeye nursery lake in BC (Simpson et al. 1981; Shortreed et al. 2001).

In an attempt to boost sockeye production, Fisheries and Oceans Canada (DFO) deposited aqueous ammonium nitrate and ammonium phosphate into the lake weekly during the growing season from 1979 to 1983 and in 1985 (1979–

Fig. 1. Kitlope Lake: (*a*) north- (downstream-) facing view of Kitlope Lake showing the inlet of the Tezwa River, the lake's primary tributary (photo: Myron Kozak, c/o The Kozak Collection, 9293 Adams Road, Smithers, BC V0J 2N7, Canada); (*b*) Landsat image showing Kitlope Lake (1), glacially coloured (greyish) Gardner Canal (2), and Kemano weather station (3) (image courtesy of Natural Resources Canada); (*c*) reference map; and (*d*) bathymetric map of Kitlope Lake showing the coring location (adapted from Stockner et al. 1993).



1981, 7.67 mg P·m^{-2}·week^{-1}, 50 mg N·m^{-2}·week^{-1}; 1982–1985, 4 mg P·m^{-2}·week^{-1}, 25 mg N·m^{-2}·week^{-1}; Stockner et al. 1993; Ken Shortreed, DFO, 4222 Columbia Valley Hwy., Cultus Lake, BC V2R 5B6, Canada, personal communication, 2005).

Sockeye salmon spawn in the floodplain reaches of the Tezwa subbasin upstream of the lake, in the lake outlet river, and on several alluvial fans in the lake proper. Kitlope-bound sockeye are harvested in coastal mixed-stock commercial fisheries and by Haisla subsistence fishers in the coastal approach waters, in Kitlope Lake, and in the lower Kitlope River. Predatory harbour seals (*Phoca vitulina*) are notably abundant throughout the fjord, estuary, lake, and accessible spawning areas during the migration and spawning seasons.

DFO's Management Target Escapement (MTE) for Kitlope sockeye is 22 000 fish (Shortreed et al. 2001) and is based on the photosynthetic rate model described in Shortreed et al. (2000). Despite the pristine nature of the ecosystem, estimated annual returns of sockeye salmon to

Table 1. General limnological information for Kitlope Lake.

	1978 (unfertilized)		1979–1980 (fertilized)	2005
Total nitrogen ($\mu g \cdot L^{-1}$)	—		—	121.4
Mean epilimnetic nitrate ($\mu g \cdot L^{-1}$)	19		18	48
Total phosphorus ($\mu g \cdot L^{-1}$)	1.5–8.0		—	7.1
Soluble reactive phosphorus ($\mu g \cdot L^{-1}$)	<1		<1	2.0
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	0.56		0.60–1.10	0.96
Total alkalinity (mg $CaCO_3 \cdot L^{-1}$)	1.6		1.5	—
Daily photosynthetic rate (mg $C \cdot m^{-2}$)	54		65	—
Zooplankton biomass (mg dry weight $\cdot m^{-2}$)	53		88	—
Euphotic zone (m)	7.6		8.6	9.7
Secchi disk depth (m)		1–6		1.3–4.2
Turbidity (NTU)	—		—	2.18
Seasonal average surface temperature (°C)		10.5		15.3
pH	6.22		6.10	6.46
Specific conductance (μS)	—		—	8.21
Drainage basin area (km^2)	872			
Lake area (km^2)	11.9			
Estimated average residence time (years)	0.49			
Estimated daily surface layer flushing (%)	15–250			
Depth (m)	86 (mean), 140 (maximum)			
Elevation (approximate) (m above sea level (a.s.l.))	13			

Note: The 1978–1980 data are means from weekly sampling through the growing season taken from Stockner et al. (1993); our 2005 data are means from four sampling events (5 July, 2 August, 23 August, 26 September) for N and P and two additional sampling events (30 May, 18 June) for all other variables.

Kitlope Lake have averaged <10 000 over the last decade and have not consistently exceeded the MTE since the late 1960s (DFO BC16 database; Spilsted and Spencer 2009).

Sediment collection and analysis

We obtained a suite of cores from the middle of Kitlope Lake on 25 June 2005 (120 m depth; 53°6.95′N, 127°46.90′W; Fig. 1*d*) using a portable percussion coring device (Gilbert and Glew 1985) with 2.0 m Lexan® tubes, fitted with brass shimstock core catchers (Reddering and Pinter 1985) and driven using a 22.7 kg hammer. Cores were transported vertically to a nearby field camp and sectioned at 2.5 mm intervals using a vertical extruder (Glew 1988). Samples were placed in Whirlpak® bags and transported on ice and in the dark to the Flathead Lake Biological Station (Polson, Montana, USA), where they were stored under nitrogen gas in the dark at 4 °C. Following rationale of Brock et al. (2006), a single 80 cm core was selected for study; the rest were maintained as backups.

Samples were weighed, homogenized with a stir rod, and subsampled under minimal lighting. For fossil pigment analysis, frozen subsamples were transported to the University of Regina Limnology Laboratory (Regina, Saskatchewan, Canada) in dark, air-tight containers flushed with N_2 gas. Subsamples for radioisotope, stable isotope, and loss-on-ignition (LOI) analyses were oven-dried at 60 °C. We assumed that sedimentary inorganic carbon concentrations were negligible because calcium carbonate concentrations are near detection in Kitlope Lake (Table 1) and no gas evolved from random sediment subsamples treated with hydrochloric acid (HCl). Given that acid-washing has been shown to affect stable isotope ratios in young sediments (Meyers and Teranes 2001), we did not treat any samples with HCl prior to analyses.

Sediment chronology

Sediment deposition dates were estimated by Flett Research Ltd. (Winnipeg, Manitoba, Canada) from down-core declines in ^{210}Pb activity using the constant rate of supply (CRS) model (Appleby and Oldfield 1978). Based on the methodology of Eakins and Morrison (1978), ^{210}Pb activities were measured (via ^{210}Po) on 13 sections using an Ortec "Octet" alpha spectrometer. All counting times were 60 000 s. Background ^{210}Po was estimated by measuring ^{226}Ra using ^{222}Rn emanation (Mathieu et al. 1988) on three deeper sections where ^{210}Po appeared to be above background levels.

Carbon and nitrogen analyses

Subsamples for elemental and isotopic N and C analyses were processed in a random order and dry-sieved at 250 μm to remove terrestrial macrofossils; mean (± standard deviation, SD) subsample weights were large (51.528 ± 1.00 mg) to ensure that elemental and isotopic measurements would be above detection limits. Total concentrations and stable isotope ratios of carbon and nitrogen were measured at the University of California Davis Stable Isotope Facility (Davis, California, USA) by pyrolysis and gas chromatographic separation in an elemental analyzer followed by isotopic analysis in a continuous-flow isotope ratio mass spectrometer. Stable nitrogen and carbon isotope ratios were, respectively, expressed as

$$(1) \quad \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000, \; R = {}^{15}N/{}^{14}N$$
$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000, \; R = {}^{13}C/{}^{12}C$$

Coefficients of variation for checks (n = 37) done on the working standards for N, $\delta^{15}N$, C, and $\delta^{13}C$ were 1.22%, 11.87%, 1.70%, and 0.14%, respectively. Total organic carbon (TOC) and total nitrogen (TN) values were calculated

as a percentage by mass and organic carbon to nitrogen ratios (C:N) were calculated as mass (not atomic) ratios.

Sediment organic matter and fossil pigments

Sediment organic matter content was estimated from percentage LOI (500 °C for 2 h). Fossil pigments in the sample set were quantified with high-performance liquid chromatography (HPLC) (Leavitt and Hodgson 2001). Carotenoid, chlorophyll, and derivative-compound concentrations were expressed as nanomoles per gram of organic matter. Analysis was restricted to chlorophylls *a*, *b*, and *a'*, phaeophytins *a* and *b*, and the following carotenoids: alloxanthin (indicator of cryptophytes), diatoxanthin (mainly diatoms), fucoxanthin (silaceous algae and some dinoflagellates), lutein + zeaxanthin (chlorophytes and cyanobacteria), and *β*-carotene (all algae) (Leavitt and Hodgson 2001). All pigments were highly correlated with each other throughout the upper 25 cm of the core; so to simplify the analysis, we derived two fossil pigments time series: (*i*) the sum of all carotenoids as an index of total algal production, and (*ii*) the ratio of chlorophyll *a* to phaeophytin *a* (chl–phaeo ratio) as a metric of postdepositional pigment degradation or transformation (Leavitt and Hodgson 2001).

Data analysis

The majority of years sampled were represented by two or three core sections owing to the high sedimentation rate in Kitlope Lake. Therefore, seasonal variation was evident in elemental and isotopic N and C time series. As our objective was to focus on interannual patterns of variation, each section was first assigned a median decimal year (e.g., 1965.2) based on the modeled ^{210}Pb ages (see Results) and grouped into 1-year bins for graphical representation and 3-year bins for numerical analyses. Because of especially high interannual variation in the δ^{15}N time series, an additional time series was created by applying a five-section (1.25 cm) running average to the δ^{15}N data prior to binning. We also constructed 3-year bins for salmon and climate time series. Such high-frequency filters helped to compensate for potential errors in the ^{210}Pb chronology, as well as those in the sockeye escapement estimates. Finally, we restricted our analysis to the period 1958–2005 because of unreliable sockeye salmon escapement estimates in earlier data (DFO unpublished area history reports and BC16 files, described in Spilsted and Spencer 2009).

Time series for each variable were assessed for normality (Shapiro–Wilks $\alpha = 0.05$) and were transformed as needed using SPSS software (version 12; SPSS Inc., Chicago, Illinois, USA). Of all variables, only sockeye escapement estimates were non-normal and thus were $\log_{10}$-transformed. Time series for each variable were assessed for temporal autocorrelation at $n/4$ lags (Box and Jenkins 1970), and serial dependency was considered problematic if the probability of the Box–Ljung statistic was >0.05. Significant autocorrelation in the variables TN, TOC, LOI, δ^{13}C, and PDO was removed using first-difference transformation. The TN and TOC values were divided by corresponding LOI to render TN and TOC concentrations comparable with the fossil pigment concentrations, which were expressed as nanomoles per gram organic matter (LOI). This conversion had the added effect of significantly reducing serial correlation in the untransformed TN and TOC time series and was justified because preliminary analysis of sediments revealed only trace levels of both inorganic C and N.

Pearson's correlation coefficients were calculated for both raw and first-differenced time series in 3-year bins of the following variables: TN, δ^{15}N, TOC, δ^{13}C, C:N, LOI, total carotenoids, sockeye escapements, mean annual temperature, mean annual precipitation, and mean annual PDO index values. For these calculations, the most recent 6 years (~3 cm) of data were excluded as a sharp decline in chl–phaeo ratios in this region of the core suggested diagenesis of sedimentary organic matter during the past decade (e.g., Leavitt 1993; Meyers et al. 1995). Additional calculations of Pearson coefficients were performed on other time series, including first-difference transformed mean annual temperature and PDO values, cross correlations between PDO, temperature, and sockeye escapements at multiple-year lags, as well as alternative treatments of 3-year binned series listed above. Correlation coefficients were not calculated between raw and differenced time series, and correlations were not corrected for the number of comparisons (e.g., Bonferroni correction).

Finally, we used sedimentary δ^{15}N values to estimate the fraction of ecosystem N uniquely derived from marine sources using a two-source mixing model following Schindler et al. (2005*a*, 2006). The model used salmon escapement data to estimate terrestrial N influx to Kitlope Lake and then calculated the importance of MDN in the overall nutrient budget of the lake. The average background loading of N from allochthonous sources (N_{ws}, as kg N·m^{-2}) to the lake since 1958 was estimated as

$$(2) \qquad N_{ws} = \frac{N_{sal}\,(\delta^{15}N_{sal} - \delta^{15}N_{sed})}{(\delta^{15}N_{sed} - \delta^{15}N_{ws})}$$

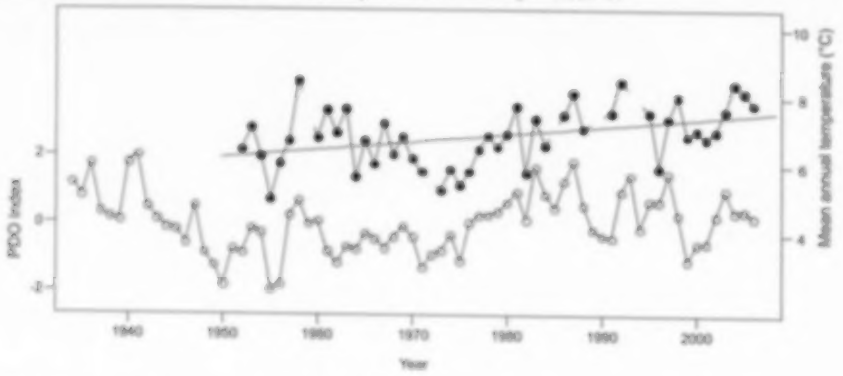
where N_{sal} is the mean annual influx of N based on observed escapement of salmon since 1958 (as kg N·km^{-2}), $\delta^{15}N_{sal}$ is the mean N (±SD) isotope ratio of adult salmon (11.2‰ ± 0.45‰; Schindler et al. 2005*a*), $\delta^{15}N_{sed}$ is the mean sedimentary N ratio since 1958, and $\delta^{15}N_{ws}$ is a range of average N isotope values from regional reference lakes (0.0‰ ± 1.0‰; L. Bunting, P.R. Leavitt, and D.E. Schindler, unpublished data). This equation was solved to derive a series of possible N_{ws} values for Kitlope Lake, which was then used to calculate the relative importance of N from marine sources during the period of record as % MDN = $N_{sal}[N_{sal} + N_{ws}]^{-1} \times 100$. This method assumed that minimal variation in the N isotopic signatures of allochthonous sources ($\delta^{15}N_{ws}$) occurred, that isotopic signatures of sediments did not exceed that of spawning salmon (e.g., due to nitrification or denitrification), and that minimal processing of δ^{15}N followed deposition in the sediments (Teranes and Bernasconi 2000).

Results

Climate and sockeye salmon dynamics

Analysis of historical time series revealed a local warming trend near Kitlope Lake (Fig. 2), a pattern that was supported by the qualitative observations by Haisla and other local people that the once regular freeze-ups of Gardner Ca-

Fig. 2. Mean annual air temperature (solid circles) at Kemano (53°33.8′N, 127°56.9′W, elevation 87 m above sea level), data from Environment Canada (2007); and mean annual Pacific Decadal Oscillation (PDO) index values (open circles), data from the Joint Institute for the Study of Atmosphere and Oceans (JISAO, University of Washington and National Atmospheric and Ocean Administration) (2007); $r_{temp\text{-}PDO}$ = 0.48, $P < 0.001$, for first-difference residuals. Magnitude of temperature trendline: $y = 0.0234x$.



nal (near Kemano; Fig. 1*b*) have nearly ceased over the past three decades. The first-difference residuals of the PDO index were significantly correlated ($r = 0.48$, $P < 0.001$) with mean annual air temperature residuals at lag 0 for the period 1952–2006 (Fig. 2), but correlations at all other lags were not significant ($P > 0.05$). Cross correlations between residuals for sockeye escapements and temperature (1958–2005) were significant but not strong at lag 0 ($r = 0.29$, $P < 0.05$) and lag –5 ($r = 0.39$, $P < 0.01$), but not for other lags tested. Cross correlations were not significant between PDO and sockeye escapements at any lag. Mean annual precipitation was not significantly correlated with PDO, temperature, or sockeye escapements, nor was it correlated with any of the sedimentary proxies (Table 2).

Sediment chronology

We documented an exponential decline in ^{210}Pb activity as a function of depth in the core (Fig. 3), a pattern that suggests few unexpected changes in sediment accumulation rate. The surface total activity was ~15 times greater than the ^{210}Pb activity (~0.80 disintegrations per minute (DPM)·g^{-1}) at 65.5–65.75 cm depth. Analysis of ^{226}Ra in depths of 55.5–55.75 cm (0.86 DPM·g^{-1}), 65.5–65.75 cm (0.92 DPM·g^{-1}), and 75.25–75.5 cm (2.28 DPM·g^{-1}) confirmed that the lowest ^{210}Pb activity was the likely background level. Based on these analyses, the CRS calculation showed that the sediment accumulation rate in the upper ~25 cm of the core averaged 4.7 mm·year^{-1}, likely reflecting the high influx of glacial silt in the terrestrial runoff (Stockner et al. 1993).

Carbon and nitrogen storage and isotopes in Kitlope Lake bulk sediments

Average values for TN and TOC were extremely low in Kitlope Lake bulk sediments (0.07% ± 0.02% and 1.16% ± 0.25%, respectively). Values fluctuated throughout the upper 25 cm of the core and generally decreased with core depth (Fig. 4). The relationship between TN and TOC was linear, with a negative TN intercept (TN = 0.07(TOC) – 0.016; $r = 0.89$, $P < 0.001$ for first-difference transformed residuals), demonstrating that concentrations of inorganically bound nitrogen were negligible and allowing us to compare TN with TOC as measured (Talbot 2001). First-differenced sediment organic matter concentrations were significantly correlated with TOC ($r = 0.79$, $P = 0.001$) and TN ($r = 0.73$, $P = 0.002$), further indicating that the bulk of sedimentary C and N was associated with organic matter.

Sedimentary δ^{15}N values were extremely low ($\bar{x}$ = –0.28‰ ± 0.79‰), consistent with low MDN contribution from salmon (~11.3‰; Schindler et al. 2005*a*). The observed C–N ratios ($\bar{x}$ = 16.77 ± 1.44) suggested contributions of organic matter from both terrestrial and aquatic sources in the sediments (Meyers and Teranes 2001). However, both δ^{15}N and the C–N ratio exhibited marked fluctuations downcore (respective ranges: 4.14‰ and 7.1), indicating that the relative balance of autochthonous and allochthonous matter varied during the study period 1958–2005 (Fig. 4). In addition, sedimentary TN, TOC, and δ^{15}N all increased sharply in the most recent ~5 years (Fig. 4). In contrast, sedimentary δ^{13}C fluctuated less than the other fossil proxies and generally increased with depth (Fig. 4).

The C:N time series was negatively correlated with LOI-corrected TN ($r = –0.78$, $P = 0.001$) but not with LOI-corrected TOC ($r = –0.28$, $P = 0.33$) (Table 2), implying that the increase in the C–N ratio with depth (Fig. 4) was a result of a proportionately greater decrease in TN than in TOC. Further, the strong negative correlation between δ^{15}N and the C–N ratio (raw, $r = –0.50$, $P = 0.07$; first-differenced, $r = –0.85$, $P < 0.001$) showed that when the organic C–N ratio was low, both sedimentary N concentration and the δ^{15}N signatures were elevated.

Sockeye-derived MDN appeared to influence sedimentary δ^{15}N and the C–N ratio, but other factors, particularly climate, apparently were more important, especially after ca. 1975. For example, the highest δ^{15}N values in the core coincided with the highest sockeye escapements on record, when time series were binned at 3-year intervals and with an offset of 1.5 years for annually resolved data (Fig. 4). Sedimentary δ^{15}N was highly correlated with sockeye escapements during 1958–1975 ($r = 0.89$, $P = 0.02$ for first-difference residuals), but not from 1958–1999 ($r = 0.39$, $P = 0.17$), unless using the smoothed δ^{15}N time ser-

Table 2. Pearson correlation matrix for sedimentary, climate, and sockeye escapement time-series data (raw and first-differenced) for samples describing the period 1958–1999.

	TN (%)	$\delta^{15}N$	$\delta^{15}N$ smooth	TOC (%)	$\delta^{13}C$	C–N ratio	LOI (%)	Carotenoids	Sock. esc.	Temp.	Precip.	PDO
TN (%)	—	0.23	0.31	0.80‡	*–0.44*	–0.78‡	*0.29*	0.70‡	*–0.09*	0.05	*0.04*	*0.22*
$\delta^{15}N$	0.39	—	0.92‡	*–0.07*	*0.18*	*–0.50**	*0.07*	*–0.09*	0.39	0.57†	*0.16*	*0.02*
$\delta^{15}N$ smooth	0.47*	0.92‡	—	0.10	*0.21*	*–0.43*	*–0.02*	*–0.02*	0.59†	0.47*	*0.19*	*–0.06*
TOC (%)	0.89‡	0.04	0.13	—	*–0.28*	*–0.28*	*0.02*	0.79‡	*–0.08*	*–0.28*	*0.14*	*–0.02*
$\delta^{13}C$	*–0.40*	0.02	0.02	*–0.38*	—	*0.30*	*–0.48**	*–0.70‡*	*0.31*	*–0.07*	*0.01*	*–0.15*
C–N ratio	*–0.39*	*–0.85‡*	*–0.84‡*	0.05	0.13	—	*–0.34*	*–0.29*	0.02	*–0.28*	*0.13*	*–0.31*
LOI (%)	0.73‡	*–0.03*	*–0.11*	0.79‡	*–0.37*	0.07	—	*0.29*	*–0.45*	*0.52**	*0.18*	*0.62**
Carotenoids	0.68‡	0.08	0.22	0.74‡	*–0.66†*	*–0.09*	0.38	—	*–0.22*	*–0.16*	*–0.01*	*0.05*
Sock. esc.	*–0.11*	0.40	0.56†	*–0.34*	0.05	*–0.53**	*–0.61†*	*–0.02*	—	0.16	*0.24*	*–0.40*
Temp.	*–0.09*	0.54†	0.37	*–0.21*	0.33	*–0.23*	*–0.13*	*–0.26*	0.28	—	*0.37*	*0.57**
Precip.	*–0.13*	*–0.06*	*–0.12*	0.09	0.16	0.37	*–0.06*	0.05	0.06	0.36	—	*–0.11*
PDO	*–0.56**	*–0.20*	*–0.35*	*–0.39*	0.53*	0.45	*–0.15*	*–0.53**	*–0.22*	0.49*	0.20	—

Note: Italics indicate significant temporal autocorrelation in at least one of the two time series. Raw (but not differenced) total N (TN) and total organic C (TOC) values were divided by percent organic matter (OM, as determined by loss-on-ignition (LOI) analyses) to reduce serial correlation and facilitate comparison with OM-specific carotenoid concentrations. Coefficients in the upper right represent raw time series; coefficients in the lower left represent first-difference transformed time series. Sock. esc., sockeye escapement; Temp., temperature; Precip., precipitation; PDO, Pacific Decadal Oscillation.

*$P < 0.10$.

†$P < 0.05$.

‡$P < 0.01$.

Fig. 3. Depth profile of ^{210}Pb activity measurements (solid circles) for Kitlope Lake in the sediment core. The three ^{226}Ra measurements (open circles) confirmed that the increased ^{210}Pb activity in the deepest measurement was likely due to increased ^{226}Ra.

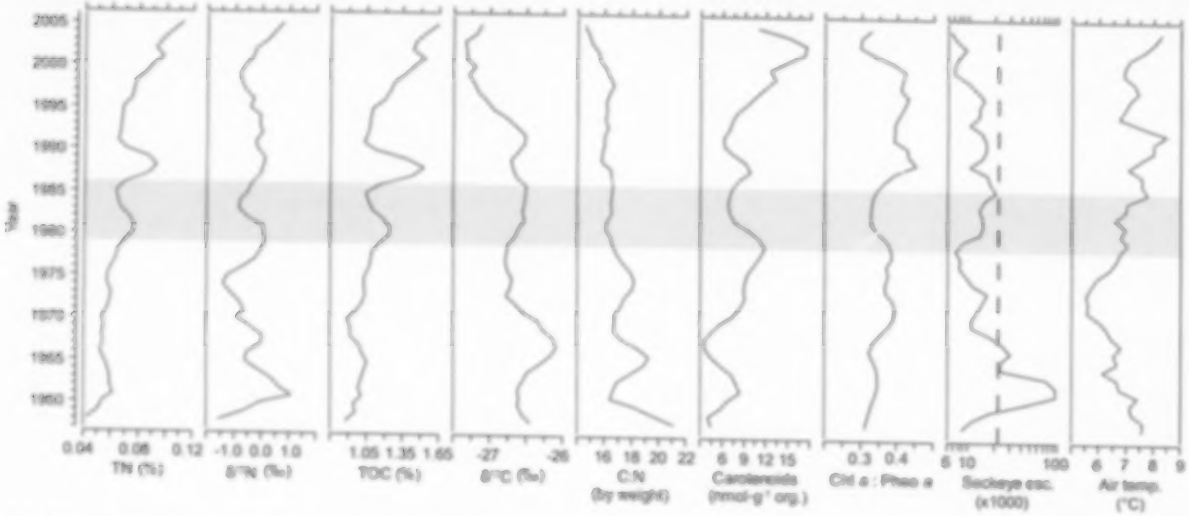
ies ($r = 0.59$, $P < 0.03$). Mean annual air temperatures were significantly correlated with $\delta^{15}N$ during 1958–1999 ($r = 0.57$, $P = 0.03$; Fig. 4). The C–N ratio inversely tracked $\delta^{15}N$ in the lower and upper portions of the core and to a lesser extent through the rest of the core (Fig. 4). Finally, the first-differenced C–N ratio and sockeye time series were marginally correlated ($r = -0.53$, $P < 0.1$; Table 2), and the two-source mixing model indicated that on average ≪2% of total N in Kitlope Lake was of marine origin.

Sediment organic matter and fossil pigments

Mean (±SD) sediment organic matter (OM) content was very low (3.75% ± 0.56%) and generally stable over the past 48 years with two exceptions: a spike from about 1987–1989 and a steady increase in the most recent 5 years that may reflect early diagenesis. Both OM anomalies were apparent in the TN and TOC curves (Fig. 4). The strong coincidence of elevated C–N ratios and OM content at ca.1987–1989 is consistent with an influx of terrestrial OM and with the presence of abundant terrestrial macrofossils in these sections (9.5–10.5 cm depth) observed during sample preparation.

Ratios of chlorophyll *a* to phaeophytin *a* were low (0.3–0.4), uncorrelated with total carotenoids ($r = 0.10$, $P = 0.73$), and relatively stable over the top 25 cm of the core (Fig. 4), suggesting a constant preservation environment following deposition (Leavitt 1993). Despite apparently good preservation, concentrations of fossil carotenoids were very low and fluctuated substantially over the past 48 years ($\bar{x} = 8.61 \pm 3.52$ nmol·(g OM)$^{-1}$), with minimum levels in the deepest sediments and an overall increase towards surficial sediments (ca. 2003). Concentrations of fossil carotenoids were highly correlated with LOI-corrected TN ($r = 0.70$, $P = 0.005$), LOI-corrected TOC ($r = 0.79$, $P = 0.001$; Table 2), and $\delta^{13}C$ ($r = -0.66$, $P < 0.01$ for first-difference re-

Fig. 4. Comparison of ^{210}Pb-dated sedimentary time-series productivity proxies from Kitlope Lake with sockeye salmon escapement estimates ($\log_{10}$ scale) and mean air temperatures. All time-series data except δ^{13}C were smoothed with a 3-year running average. The shaded area indicates the period of artificial fertilization, and the vertical broken line indicates the management target sockeye escapement of 22 000 fish.



sidual), consistent with a slow increase in overall lake production during the study period (Table 2). Fossil carotenoids were not correlated with sockeye salmon escapements ($r = -0.22$, $P = 0.45$), although the highest carotenoid concentrations from ca. 1958–1968 coincided with the 1963 record high sockeye escapement (Fig. 4), implying that greatly elevated spawner densities MDN may have occasionally influenced aquatic primary production (Schindler et al. 2005*a*; Brock et al. 2007).

Discussion

Mean δ^{15}N for Kitlope Lake was the lowest yet reported from a sedimentary time series derived from a sockeye salmon nursery lake worldwide. On average, recent marine nitrogen (δ^{15}N) contributions to total Kitlope Lake N were trivial (≪2‰); however, sedimentary δ^{15}N, organic C:N, and fossil carotenoids all appeared to respond to the order-of-magnitude increase in spawner density ca. 1961–1964, suggesting that episodic influx of MDN could stimulate lake production. In addition, the timing of changes in the correlation structure of sedimentary time series suggested that control of lake production shifted from occasional influence of MDN and that the recent warming trend coincided with a proxy-inferred increasing trend in lake production. The C–N ratio indicated a substantial terrestrial contribution to sediment organic matter that is decreasing relative to autochthonous sources according to both the δ^{13}C and C:N signatures. Therefore, trophic status in this system, and likely in similar systems, is driven by climate, catchment sources of organic matter, and salmon import, as well as in-lake productivity.

Climate and salmon trends

As expected, local mean annual air temperatures were significantly and positively correlated with mean annual Pacific Decadal Oscillation (PDO) index values, and a local warming trend was evident starting in ca. 1976, the period at which the PDO shifted to a warming phase (Mantua and Hare 2002). In the case of Kitlope sockeye, inconsistencies in historic catch data precluded calculations of overall stock production, and the escapement data did not support interpretation of events involving only a single year. With these caveats, we note that positive correlations between mean annual air temperatures and sockeye escapements were significant only at the 0 and –5 year lags consistent with the finding that the majority of Kitlope sockeye return to spawn at 5 years of age (Rosberg et al. 1982). This result, combined with the absence of significant correlations between sockeye escapements and PDO, may indicate that population variability is more strongly entrained to local climatic conditions than to variation at the scale of the North Pacific Ocean. This contention is also supported by the observations that spatial synchrony in sockeye production is stronger within regions than among regions (Peterman et al. 1998), that environmental processes exert more control on sockeye survival at local and regional (<500 km) scales than at oceanic scales (Pyper et al. 2005), and that coastal sea surface temperatures are often correlated with sockeye survival during freshwater residency (Mueter et al. 2005). Finally, the lack of a strong relationship between the PDO and variables other than temperature may reflect the proximity of the Kitlope region to the Subarctic Boundary transition zone separating the major northern and southern production domains of the Northeast Pacific. These zones often respond biologically in opposite directions to PDO-scale forcing, whereas ecosystem response in the transition zone is often less categorical, relative to the PDO (Francis et al. 1998).

Historical changes in nursery lake production

Our 1958–2005 sedimentary record confirmed the ultra-oligotrophic status of Kitlope Lake, consistent with the ex-

tremely low ambient concentrations of nutrients, rapid flushing rate, and the strong influence of the lake's main tributary, the glacially turbid Tezwa River. Sedimentation rates were remarkably high in Kitlope Lake (4.7 mm·year^{-1}), and sediments contained extremely low concentrations of total nitrogen (TN) and organic carbon (TOC). The mean (±SD) C–N ratio (16.8 ± 1.4) indicated that terrestrial sources substantially contributed to bulk sediment organic matter, as cellulose-rich terrestrial primary producers generally have much higher organic C:N (>~20) than do protein-rich lake algae (<~10) (Meyers and Teranes 2001). Elevated influx of allocthonous matter is further indicated by the predominance of periphytic rather than planktonic diatoms in midlake epilimnetic phytoplankton samples (Stockner et al. 1993). Moreover, very few diatoms in our core were of planktonic origin (2% *Tabellaria* sp., n = 10; A.C. Hill, unpublished data), although diatom preservation was generally poor, making it difficult to recover adequate numbers of frustules for a more thorough quantitative analysis of community composition. In general, analyses of most sedimentary proxies (OM, fossil carotenoids, TN, TOC, C:N) were consistent with extremely low algal production. Nonetheless, these variables were generally correlated with one another and, along with sedimentary $\delta^{13}C$ (discussed below), indicated an overall increase in algal abundance during the period 1958–2005.

Influence of temperature and MDN on algal production

In P-limited nursery lakes such as Kitlope Lake, there is often an approximately linear relationship between sockeye escapements and sedimentary $\delta^{15}N$ (Finney et al. 2000; Brahney et al. 2006; Brock et al. 2007). In Kitlope Lake, sockeye escapements were significantly correlated with $\delta^{15}N$ and with productivity proxies (C:N, fossil carotenoids) over the period 1958–1975, but less so over the 1958–1999 study period. Significant correlations were surprising given the small (≪2%) average contribution of marine N to the total N budget indicated by the mixing model and contrast with the findings of Holtham et al. (2004), who suggested that sockeye nursery lakes with high flushing rates and substantial terrestrial inputs were not strongly influenced by MDN flux. The apparent lake response to MDN was also surprising given that the mean sedimentary $\delta^{15}N$ in Kitlope Lake was the lowest yet reported for a sockeye salmon nursery lake (e.g., Holtham et al. 2004; Brahney et al. 2006; Hobbs and Wolfe 2008). In fact, our mean sedimentary $\delta^{15}N$ was low even compared with lakes with and without salmon in other multilake studies (Finney et al. 2000; Brock et al. 2007). We hypothesize that these apparent discrepancies may reflect the predominant role of P in regulating algal production in this lake, given that the system is severely P-limited ($N:P_{total}$ = 43, $N:P_{dissolved}$ = 53, n = 4) relative to the N–P ratio of P-rich salmon carcasses (~8:1; Larkin and Slaney 1997). In such instances, lake production could be strongly limited by allochthonous P influx from the catchment such that episodic increases in sockeye escapements are sufficient to elevate algal abundance for brief periods of time (Brock et al. 2007). Unfortunately, full mass balances of P flux were not available for Kitlope Lake; therefore, further research is required to resolve this issue.

Lake production responses to climate warming are variable among ecosystems (Leavitt et al. 2003; Karlsson et al. 2005; Schindler et al. 2005*b*) and poorly understood in the case of midlatitude, glacially turbid systems like Kitlope Lake (Melack et al. 1997). In our study, temperature appeared to replace salmon abundance as the dominant control on sedimentary $\delta^{15}N$ in Kitlope Lake concomitant with the ca. 1976 PDO shift to a warming phase (Mantua and Hare 2002). In turn, the strong correlation between $\delta^{15}N$ and C:N coupled with decreasing trends in sedimentary $\delta^{13}C$ (see below) and C:N and increasing TN, TOC, and fossil carotenoids is indicative of increasing algal production and (or) decreasing terrestrial organic matter imports from upstream sources (Leavitt 1993; Meyers and Teranes 2001; Bunting et al. 2007). These inferred trends are supported by the average chlorophyll *a* concentrations that we recorded in 2005 that were greater than those measured during the first two years of the lake enrichment program (1979–1980). Increased primary production is expected to increase the forage base for juvenile sockeye, increasing young-of-the-year (YOY) survival and smolt size (Hyatt and Stockner 1985), although not necessarily increased adult returns if other factors limit survival (e.g., Mueter et al. 2005).

Additional controls on sedimentary N and C isotopes

The significant correlation between temperature and $\delta^{15}N$, especially apparent in the latter half of the study period, was unexpected and suggests a potential effect of climate warming on the N cycle in this system. This observation may be explained by the fact that microbial activity and N cycling (i.e., nitrification and denitrification) generally increase as temperatures warm (Wetzel 2001), potentially leading to increased isotopic fractionation for a given N pool size (Talbot 2001). Given the extremely low sedimentary $\delta^{15}N$ values observed even under high MDN loading (e.g., ca. 1963), even moderate temperature-induced increases in N cycling rates may be expected to register in the sedimentary $\delta^{15}N$ record owing to the extreme P limitation.

We are not aware of any paleoecological studies to date that have used $\delta^{13}C$ to infer changes in organic matter source over time in a sockeye salmon nursery lake. However, the observed decrease in sedimentary $\delta^{13}C$ over time was likely indicative of an increasing algal contribution to the bulk sediments, as sedimentary $\delta^{13}C$ from Kitlope Lake was negatively correlated with fossil carotenoids, similar to patterns observed in temperate lakes by Bunting et al. (2007). Lake algae have significantly lower $\delta^{13}C$ values than terrestrial particulate organic matter in arctic lakes (Kling et al. 1992), and in the upper Kitlope River, the mean (±SD) $\delta^{13}C$ of perphytic algae (–32.70‰ ± 5.08‰, n = 57) was substantially lower than the foliar $\delta^{13}C$ of dominant riparian deciduous trees and shrubs (–28.97‰ ± 1.71‰, n = 101; J.A. Stanford, unpublished data). Seasonal CO_2 limitation is well known to control sedimentary $\delta^{13}C$ (Meyers and Teranes (2001) and references therein), but this was unlikely to have occurred in Kitlope Lake given its extreme oligotrophy and weak (cold) thermal stratification. Moreover, diagenetic effects on sedimentary organic $\delta^{13}C$ are known to be minimal (Meyers et al. 1995; Hodell and Schelske 1998), and Kitlope Lake sedimentary $\delta^{13}C$ appeared robust to seasonal (not shown) and annual changes in organic matter concentration.

Management implications

This study has demonstrated concurrent increases in lake algal production and air temperature likely arising from both directional planetary warming and changes in oceanic climate patterns (e.g., PDO). We further demonstrated that even with high flushing rates and high background N concentrations, algal production in this glacially turbid, ultraoligotrophic nursery lake was likely affected by the abundance of spawning sockeye salmon. If so, increased production potential related to climate warming may be offset by declining MDN influx as a result of declining escapements, suggesting that the forage base for sockeye salmon fry in Kitlope Lake may benefit from increased nutrient flux. Moreover, what little rearing capacity exists in ultraoligotrophic BC sockeye nursery lakes is often underutilized due to spawning escapements that are substantially below their theoretical optimum (Riddell 2004; Price et al. 2008). Given the known strong interaction between lake fertility and YOY survival and growth (Quinn 2005), production and survival of juvenile sockeye in Kitlope Lake may benefit by reversing the decline in escapement and maintaining escapements at or above management targets.

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Spatial and temporal spawning dynamics of native westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, introduced rainbow trout, *Oncorhynchus mykiss*, and their hybrids

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Abstract: We used radiotelemetry to assess spatial and temporal spawning distributions of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT), introduced rainbow trout (*Oncorhynchus mykiss*; RBT), and their hybrids in the upper Flathead River system, Montana (USA) and British Columbia (Canada), from 2000 to 2007. Radio-tagged trout ($N = 125$) moved upriver towards spawning sites as flows increased during spring runoff and spawned in 29 tributaries. WCT migrated greater distances and spawned in headwater streams during peak flows and as flows declined, whereas RBT and RBT hybrids (backcrosses to RBT) spawned earlier during increasing flows and lower in the system. WCT hybrids (backcrosses to WCT) spawned intermediately in time and space to WCT and RBT and RBT hybrids. Both hybrid groups and RBT, however, spawned over time periods that produced temporal overlap with spawning WCT in most years. Our data indicate that hybridization is spreading via long-distance movements of individuals with high amounts of RBT admixture into WCT streams and stepping-stone invasion at small scales by later generation backcrosses. This study provides evidence that hybridization increases the likelihood of reproductive overlap in time and space, promoting extinction by introgression, and that the spread of hybridization is likely to continue if hybrid source populations are not reduced or eliminated.

Résumé : La radiotélémétrie nous a servi à évaluer la répartition spatiale et temporelle de la fraie chez la truite fardée indigène du versant occidental (*Oncorhynchus clarkii lewisi*; WCT), la truite arc-en-ciel (*Oncorhynchus mykiss*; RBT) introduite et leurs hybrides dans le réseau hydrographique supérieur de la Flathead au Montana (É.-U.) et en Colombie-Britannique (Canada) de 2000 à 2007. Les truites porteuses d'une étiquette radio ($N = 125$) se sont déplacées vers l'amont vers les sites de fraie durant la crue printanière et ont frayé dans 29 tributaires. Les WCT ont migré sur de plus grandes distances et ont frayé dans les cours d'eau les plus en amont durant les débits maxima et durant la réduction des débits, alors que les RBT et les hybrides RBT (rétrocroisés avec RBT) ont frayé plus tôt durant l'augmentation des débits et plus en aval dans le bassin. Les hybrides WCT (rétrocroisés avec WCT) ont eu une fraie intermédiaire dans le temps et l'espace à celles des WCT, des RBT et leurs hybrides RBT. Cependant, les deux groupes d'hybrides et RBT ont frayé durant des périodes qui chevauchent la fraie des WCT la plupart des années. Nos données indiquent que l'hybridation se propage grâce aux déplacements sur de longues distances vers les cours d'eau à WCT d'individus qui possèdent un fort complément de RBT et par une invasion par degrés par les générations subséquentes rétrocroisées. Notre étude fournit des preuves que l'hybridation augmente la probabilité de chevauchement reproductif dans le temps et l'espace, ce qui favorise l'extinction par introgression; la propagation de l'hybridation va vraisemblablement continuer à moins que la source de populations d'hybrides ne soit réduite ou éliminée.

[Traduit par la Rédaction]

Introduction

Invasive species threaten the biodiversity of aquatic ecosystems worldwide (Mack et al. 2000) and are considered the second greatest threat to biodiversity loss in North America (Mooney and Cleland 2001). Hybridization (interbreeding of individuals from genetically distinct populations) between introduced and native taxa can be a major consequence of species introductions, especially when nonnative species hybridize with rare or endangered species, threatening their persistence (Rhymer and Simberloff 1996).

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Hybridization with introduced species has been a major factor in the decline and extinction of many native fishes throughout North America (Miller et al. 1989). Although natural hybridization can lead to evolutionary novelty and speciation in many plants and some vertebrate species (Arnold 1997; Barton 2001), human-mediated hybridization and introgression (gene flow between populations with individuals that hybridize) can lead to the extinction of native genotypes and the loss of locally adapted gene complexes and ecological adaptations (Rhymer and Simberloff 1996; Allendorf et al. 2001). Therefore, understanding the invasion patterns and mechanisms that permit and constrain hybridization is required for conservation of aquatic biodiversity and developing recovery and management programs for native species at risk of hybridization with introduced taxa.

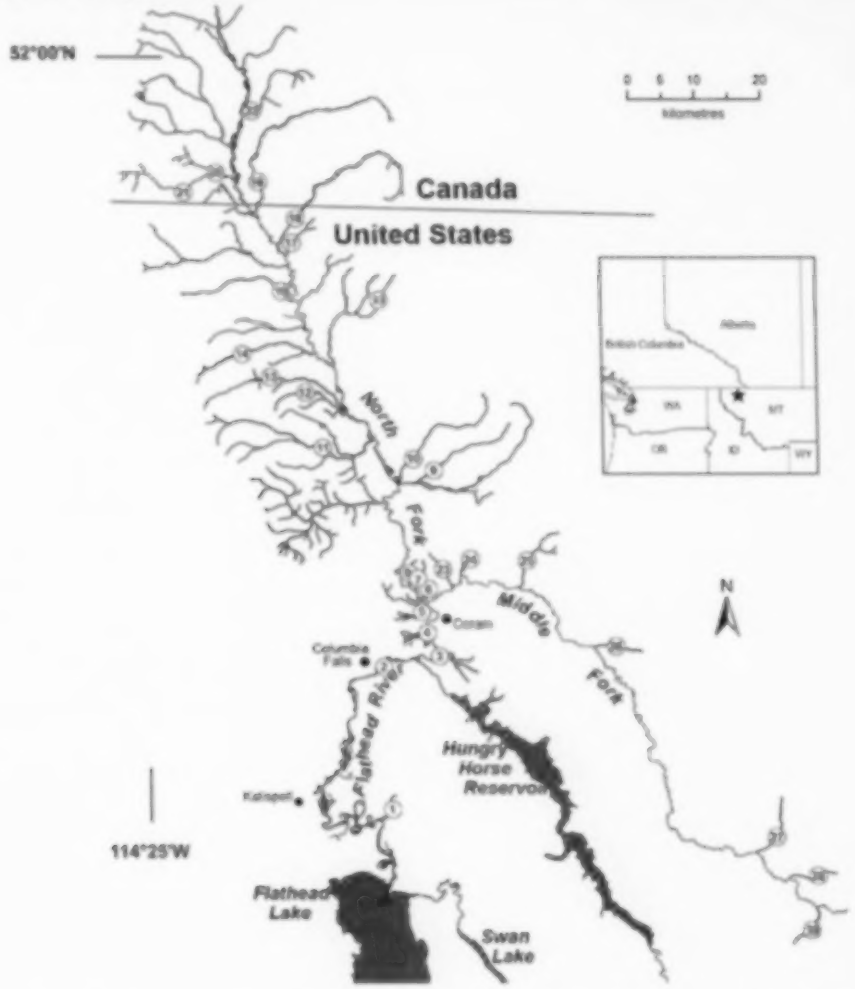
Hybridization is more common in fish than in any other vertebrate taxa (Leary et al. 1995; Allendorf et al. 2001). Many fishes have external fertilization and similar mating behaviors, facilitating interbreeding. In undisturbed ecosystems, reproductive isolation is maintained primarily by spatial and temporal reproductive isolation. However, such barriers to interbreeding are lost in the face of habitat disturbance and introduction of nonnative species (Allendorf et al. 2001). This is particularly true for salmonids in which widespread introgression among native and nonnative taxa has replaced native species over large areas throughout their native ranges, which is suspected to lead to a loss of behavioral and ecological adaptations in native populations (Allendorf and Leary 1988; Rhymer and Simberloff 1996). Reproductive behavior is intimately linked to salmonid life histories, which represent long-term adaptations to the natural environment. Thus, reproductive behavior is considered to be a critical trait influencing the persistence of native salmonid populations. The consequences of human-mediated hybridization on salmonid reproductive behavior, however, are poorly understood.

Introgressive hybridization with introduced rainbow trout (*Oncorhynchus mykiss*; RBT) is considered one of the greatest threats facing many native salmonid populations worldwide (Behnke 1992; Leary et al. 1995). Widespread transplanting of RBT outside their native range has caused local extirpations or reductions of native salmonids in nearly all drainages in which they have been introduced, and the rate of introductions has dramatically increased over the past 50 years due to intentional and unintentional releases (Fuller et al. 1999). Along with habitat loss and degradation, introgressive hybridization with RBT has played a major role in the demise of all subspecies of cutthroat trout (*Oncorhynchus clarkii*; Allendorf and Leary 1988; Gresswell 1988; Young 1995). Hybridization and introgression with introduced RBT threatens the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT) with genomic extinction, the loss of genotypes across the entire genome that are permanently lost by hybridization (Allendorf and Leary 1988; Allendorf et al. 2004). Westslope cutthroat trout are highly divergent from the other major cutthroat trout subspecies (coastal (*Oncorhynchus clarkii clarki*), Yellowstone (*Oncorhynchus clarkii bouvieri*), and Lahontan (*Oncorhynchus clarkii henshawi*) cutthroat trout; Loudenslager and Gall 1980; Leary et al. 1987; Allendorf and Leary 1988), and their geographic range is the largest of the 13 interior subspecies, encompassing the Columbia, Fraser, Missouri, and Hudson Bay drainages of the United States and Canada (Behnke 1992). Westslope cutthroat trout historically occupied a wide variety of aquatic habitats, from small headwater streams to large rivers and lakes. However, native populations have dramatically declined due to habitat degradation, fragmentation, overexploitation, and hybridization and competition with nonnative trout. Consequently, nonhybridized populations of WCT currently inhabit less than 10% of their historic range in the United States (Shepard et al. 2005) and less than 20% of their historic range in Canada (Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006). Many of the remaining nonhybridized populations are restricted to headwater areas above upstream dispersal barriers (Shepard et al. 2005). Consequently, hybrid forms are the dominant taxa in most drainages where WCT were native.

The timing and location of breeding are the primary isolating mechanisms between naturally sympatric salmonid species. However, the rapid increase of human-mediated hybridization in many salmonid species suggests that little reproductive segregation exists between naturally allopatric taxa. Although WCT co-evolved with resident and anadromous forms of RBT in portions of the Columbia River basin (Behnke 1992), either spatial or temporal reproductive segregation has limited natural hybridization between these fishes (Brown et al. 2004; but for an exception, see Bettles et al. 2005). In contrast, many studies have shown that introductions of nonnative RBT commonly result in widespread introgression and loss of native genotypes in WCT populations (Allendorf et al. 2001; Rubidge and Taylor 2004; Ostberg and Rodriguez 2006) and other subspecies of cutthroat trout (Gresswell 1988; Young 1995). The apparent lack of reproductive isolation between the introduced and native trout is assumed to be the result of their failure to evolve isolating mechanisms in allopatry. However, few studies have tested this assumption in the natural environment.

Although the upper Flathead River system in Montana and British Columbia (Fig. 1) is considered a range-wide stronghold for WCT, the long-term persistence of nonhybridized populations in the drainage is threatened by the recent spread of nonnative RBT introgression (Hitt et al. 2003; Boyer et al. 2008; Muhlfeld 2008). The drainage presents a unique opportunity to examine spawning dynamics among hybridized and nonhybridized taxa as a full range of parentals and introgressed individuals are present in the system. Understanding the factors affecting reproductive isolation between parental and hybrids in a recently invaded system will aid in the identification of the primary reproductive mechanisms in the spread of hybridization and may identify the major sources of nonnative admixture (proportion of nonnative alleles in individuals or populations) in the system. To examine differences in reproductive behavior among RBT, WCT, and their hybrids, we used radiotelemetry in the upper Flathead River system from 2000 to 2007. We hypothesized that hybrids and RBT would spawn earlier and lower in the system compared with nonhybridized WCT, but the spawning distributions would vary spatially and temporally. Additionally, we hypothesized that hybridization is spreading by long-distance movements of individuals with high amounts of nonnative RBT admixture into nonhybridized

Fig. 1. Study area location and sample site identification in the upper Flathead River system in Montana (MT, USA) and British Columbia (Canada). Sample site codes correspond to Table 1.



WCT populations. Our objectives were (*i*) to identify RBT and hybrid source populations, (*ii*) to compare the timing, location, and movements of spawning by RBT, WCT, and their hybrids, and (*iii*) to describe the patterns of spatial and temporal overlap among parental taxa and hybrid types.

Materials and methods

Study area and populations

The upper Flathead River drainage originates in the Rocky Mountains of British Columbia, Canada, and northwestern Montana, USA, and includes Flathead Lake and the North Fork, Middle Fork, South Fork, and mainstem Flathead rivers (Fig. 1). The drainage area is approximately 18 400 km^2 and is in the headwaters of the upper Columbia River basin. We implanted prespawning fish with radio transmitters from the confluence of the Middle and North Fork rivers downstream to the mainstem near Kalispell (Fig. 1). Hydropower and flood-control operations from Hungry Horse Dam in the South Fork partially regulate the lower 69 km of the mainstem (Fig. 1), resulting in the storage of spring runoff and flow augmentation during the summer, fall, and winter months. This reach supports the highest abundances of WCT × RBT fluvial hybrids in the river and lake system (Muhlfeld et al. 2003).

WCT exhibit both resident (remaining in natal streams throughout life) and migratory life history strategies in the Flathead Lake and River system, but migratory forms are most common (Shepard et al. 1984; Liknes and Graham 1988; Muhlfeld et al. 2000). Migratory WCT rear in their natal stream for 1–4 years and then migrate downstream as subadults to Flathead Lake (adfluvial) or the mainstem rivers (fluvial). Adult WCT generally overwinter in the lower river or the lake and then migrate upstream during high spring flows and spawn in tributaries. In contrast, RBT and WCT × RBT hybrids display a fluvial life history in the mainstem (Muhlfeld et al. 2003), but little was known about their spawning behaviors prior to this study.

Table 1. The number of radio-tagged fish that spawned in each stream in the upper Flathead River system from 2000 to 2007.

		No. of radio-tagged spawners			
Code	Site name	WCT	WCT hybrid	RBT hybrid	RBT
1	Mill				1
2	Taylors		1	7	4
3	Abbot			18	28
4	Ivy			1	3
5	Rabe			4	8
6	First				1
7	Steamer			1	
8	Third		1(F_1)	2	2
9	Dutch		2	2	1
10	Anaconda		3	1	
11	Coal (NF)	1	1(F_1)		
12	Moran	1			
13	Hay	1			1
14	Red Meadow	2			
15	Akokala	2	1		
16	Tepee	3			
17	Starvation	1			
18	Kishenehn	1			
19	Sage	4			
20	Burnham	1			
21	Cauldrey	1			
22	Commerce	3			
23	Rubideau		1		
24	McDonald				1
25	Lincoln	1	1		1
26	Coal (MF)	1			
27	Granite	1			
28	Lodgepole	1			
29	Schafer	2			

Note: Sites are coded in approximate order of ascending upstream distance. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrid, WCT × RBT hybrids backcrossed to WCT; RBT hybrid, backcrosses to RBT; F_1, first-generation hybrid; NF, North Fork stream; MF, Middle Fork stream.

Timing and location of spawning

We used radiotelemetry to monitor the movements and spawning behavior of WCT, RBT, and WCT × RBT hybrids from 2000 to 2007 in the upper Flathead River system, Montana (USA) and British Columbia (Canada). In 2000, 2001, 2006, and 2007, we tagged fish that visually appeared to be WCT, RBT, and hybrid trout, whereas in 2002, 2003, and 2005, we exclusively targeted hybrids and RBT. Morphological characters used for identification were spotting pattern, body coloration, and the presence or absence of red or orange slashes below the gill covers (Behnke 1992). Prespawning fish were randomly captured in the mainstem Flathead River using jet-boat electrofishing at night during February and early March. Captured fish that met the 2% body to transmitter – body weight ratio (Winter 1983) were anaesthetized with tricaine methane sulfonate (MS-222), surgically implanted with transmitters according to the methods described by Muhlfeld et al. (2003), held for 1–5 h after tagging, and released near their capture location. We selected individuals greater than 300 mm total length in which maturing gonads could be visually identified with an otoscope through the surgical incision. Transmitters emitted a unique code in the 148.780 MHz frequency range (with 5 s burst rates), had expected battery lives of 110 days (2000–2005) and 472 days (2006–2007), and weighed 6.7 g (air weight) and 10 g, respectively. A fin clip was collected from each fish during surgery, preserved in 95% ethanol, and analyzed at the Conservation Genetics Laboratory at the University of Montana, Missoula, to estimate degree of admixture.

Radio-tagged fish were relocated three to five times per week during the spring spawning period from a jet boat, from vehicle access points along the stream, and by foot using a Lotek scanning receiver (model SRX-400, Lotek Wireless Inc., Newmarket, Ontario) equipped with an ATS three-element Yagi antenna (Advanced Telemetry Systems Inc., Isanti, Min.). Aerial surveys were conducted to locate fish that were missing for more than 1 week and to survey remote and inaccessible areas throughout the upper portions of the river system, including British Columbia (Canada), Waterton–Glacier International Peace Park, and the Bob Marshall Scapegoat Wilderness complexes (Fig. 1). When logistically possible, observers walked spawning streams to gain a more accurate location and to document redd construction. Additionally, four permanent telemetry ground stations were installed near the mouths of the North Fork, Middle Fork, South Fork, and mainstem Flathead rivers, which continuously monitored fish movements within 250 m of the antenna. Each ground station consisted of a Lotek data-logging receiver equipped with an ATS three-element directional Yagi antenna powered by a 12V deep-cycle marine battery. Georeferenced locations were obtained at each fish location using a global positioning system (GPS) unit (TSC1 Asset Surveyor, Trimble Navigation Limited, Sunnyvale, California). In a geographic information system (GIS), the point locations were overlaid on a hydrography layer for analysis of distance moved, which were calculated between consecutive locations.

We were physically unable to identify the exact timing and location of spawning for radio-tagged fish due to high and turbid flows, inclement weather conditions (which precluded some aerial surveys), and the extensive size and complexity of the stream system. Therefore, we assumed that a fish had spawned if it made a pronounced (>5 km), rapid (between consecutive relocations) movement (upriver or downriver) from its release location in the mainstem into a tributary or the mouth of a tributary or made a movement to a tributary within 5 km of its tagging location. All remaining fish were classified as nonspawners.

Spawning and movement behavior was characterized by measuring eight variables including beginning migration date, beginning migration temperature (°C), beginning migration flow ($m^3 \cdot s^{-1}$), spawning date (day of year), spawning temperature, spawning flow, migration period (days), and migration distance (km). Migration was defined as an upstream or downstream movement greater than 5 km from the fish's tagging location. The beginning date of migration for each individual fish was considered to be the median date between the day when a fish migrated and the previous location. Spawning location was defined as the farthest extent of a fish's migration. Migration distance was defined as the fluvial distance between the location from which fish began their migration and the spawning location (Henderson et

al. 2000). Migration period was defined as the time (days) from the beginning date of migration until the first relocation of the fish at or near its observed or likely spawning location. Spawning date was calculated as the midway point between migration period ending date and when it left its spawning location. Spawning period for each study group (defined below) extended from the date that the first fish spawned until the last fish spawned in a particular group. Mean daily water temperature and discharge data were obtained from the US Geological Survey station on the mainstem Flathead River near Columbia Falls, Montana.

Genetic analyses

We used paired interspersed nuclear elements polymerase chain reaction (PINE PCR) techniques to assess the hybridization status of each fish tagged from 2000 through 2003 (Appendix A, Table A1; Spruell et al. 2001; Kanda et al. 2002). We used combinations of primer pairs to evaluate specific regions ("fragments") of anonymous DNA for each taxon. Six dominant nDNA fragments were examined for RBT and seven for WCT (Spruell et al. 2001). For fish tagged from 2005 to 2007, we took advantage of a new nDNA technique that increased the ability to detect nonnative alleles in individual fish by using a combination of seven diagnostic co-dominant indel loci (*Occ34*, *Occ35*, *Occ36*, *Occ37*, *Occ38*, *Occ42*, *Om55* (Ostberg and Rodriguez 2004)) and six diagnostic co-dominant microsatellite loci (*Ssa408* (Cairney et al. 2000); *Oki10* (Smith et al. 1998); *Omm1037-1*, *Omm1037-2*, *Omm1050* (Rexroad et al. 2002); *Omy0004* (Holm and Brusgaard 1999); *Omy1001* (Spies et al. 2005)). For the PINES and indel–microsatellite data, we calculated a hybrid index (HI) for each fish (Appendix A, Fig. A1). Statistical power to differentiate between parental types and early generation hybrids is high, with seven diagnostic co-dominant markers. For example, the probability that a first-generation backcross would be misclassified as a first-generation (F_1) hybrid is less than 0.01 (Boecklen and Howard 1997). However, reliable discernment between parental types and later generation backcrosses requires many diagnostic loci (Floate et al. 1994). The hybrid index, therefore, likely overestimates parental types and underestimates the number of individuals of hybrid ancestry in populations containing later generation hybrids.

Fish were classified into four groups based on their HI (Appendix A, Fig. A1). Individuals were classified as nonhybridized WCT if they had a HI of zero using either PINEs or indel–microsatellites. Individuals were classified as nonhybridized RBT if they had a HI of 13 (PINEs) or 26 (indel–microsatellites). F_1 hybrids were those fish with a HI of 6 using PINEs (RBT phenotypes were detected at only the six loci where the presence of the fragment is characteristic of RBT) and 13 using indel–microsatellites, but were heterozygous at all loci. Post-F_1 hybrids with higher proportions of WCT than RBT alleles were classified as backcrosses to WCT (WCT hybrids) and those with a higher proportion of RBT than WCT alleles were classified as backcrosses to RBT (RBT hybrids). The two F_1s monitored during the study were grouped with the WCT hybrids because their movement and spawning behavior were similar and to increase the samples size in that category. Although fish classified as RBT have diagnostic RBT alleles at all loci, recent genetic studies in the system indicate that these individuals may have originated from a private RBT hatchery (see Discussion) or from a stream (Abbot Creek) containing a hybrid swarm with 92% RBT admixture. Therefore, some of the fish classified as nonhybridized RBT may have contained a few WCT alleles. Finally, the sample size of fish with low levels of RBT admixture was lower than the samples sizes of WCT and fish with high amounts of RBT admixture. This was due, in part, to our field identification and preferential selection of fish with morphological characteristics of parental types and high proportions of RBT admixture.

Confirmation of morphological identification

We assessed whether morphological characteristics could be used to accurately predict the genetic status of adult fish in the field. Each fish was visually classified as either a WCT or a RBT hybrid and then genotyped using PINEs or indel–microsatellites (see above) to assess the percentage of fish that were correctly classified based on their individual multilocus phenotype (PINEs) or genotype (indel–microsatellites). We used data collected for the years in which we simultaneously tagged WCT and RBT hybrid spawners and nonspawners (N = 98 fish). Molecular analyses revealed an overall correct classification of 93%; four fish that were morphologically classified as WCT were genetically identified as WCT hybrids, and three fish visually classified to be WCT hybrids were genetically identified as WCT.

We were unable to genotype 11 radio-tagged fish. Thus, the genetic status of these fish was only visually determined. Of the fish visually classified as WCT (N = 5), all were estimated to have spawned in streams containing nonhybridized WCT populations (Hitt et al. 2003; Boyer et al. 2008). Similarly, of the fish visually identified as RBT (N = 6), all were observed spawning in Abbot Creek, a stream containing individuals with high amounts of RBT admixture (92%; Boyer et al. 2008). Thus, based on the population-level genetics data and the high accuracy of correctly classifying genetic status using morphological characteristics, we believe that the visual estimates of taxonomic identity for these 11 fish were likely reliable and unbiased.

Statistical analyses

Because some of the eight dependent spawning and movement variables were significantly correlated (Appendix A, Table A2), multivariate analyses were used to simultaneously test for group differences on all the response variables, all of which met the assumptions of normality and homogeneity of variance (Zar 1996). First, we used a multivariate general linear model (GLM multivariate) to test whether there were significant effects due to genetic status (group), year, and sex, which were entered as fixed effects with interaction terms. Next, we used multivariate analysis of variance (MANOVA) to assess differences among groups for the eight spawning variables. Individual analyses of variance (ANOVAs) were used to test for differences in each spawning variable among groups, and post-hoc pairwise comparisons were conducted using Tukey's tests. For all analyses, statistical significance was determined at an alpha level of 0.05. Statistical analyses were conducted using SPSS, version 10.1.4 (Norusis 1990).

Table 2. Summary statistics for radio-tagged fish monitored in the upper Flathead River drainage from 2000 through 2007.

Group	*N*	Mean length (mm)	Mean weight (g)	Mean spawning date (month/day)	SD (days)	Mean distance (km)	SD
2000							
WCT	7	410 (51)	720 (342)	6/7	15	118	32
RBT	9	392 (24)	573 (120)	5/3	7	20	14
2001							
WCT	5	392 (39)	615 (205)	6/9	9	105	7
WCT hybrid	1	464	933	5/6		77	
RBT hybrid	7	380 (22)	527 (101)	5/11	16	19	20
RBT	9	410 (31)	666 (160)	5/6	7	13	11
2002							
WCT hybrid	1	442	920	6/3		50	
RBT hybrid	9	417 (55)	735 (313)	5/23	16	15	7
RBT	11	390 (27)	579 (140)	5/22	18	27	25
2003							
WCT hybrid	1	435	781	5/25		67	
RBT hybrid	8	410 (37)	632 (154)	5/3	14	22	33
RBT	12	410 (59)	628 (347)	5/5	15	18	17
2005							
RBT hybrid	7	413 (26)	690 (170)	4/23	10	10	4
RBT	6	423 (36)	778 (177)	4/29	13	17	9
2006							
WCT	3	474 (53)	1082 (374)	6/3	20	99	35
WCT hybrid	1	465	901	5/7		1	
RBT hybrid	3	447 (48)	974 (357)	4/17	7	5	5
RBT	2	420 (14)	624 (85)	4/26	14	18	4
2007							
WCT	12	419 (37)	776 (222)	5/29	11	109	23
WCT hybrid	7	441 (31)	760 (145)	5/11	18	48	23
RBT hybrid	2	395 (28)	588 (153)	5/12	4	50	4
RBT	2	402 (22)	541 (85)	4/30	8	16	10

Note: For mean length and mean weight, the number in parentheses is the standard deviation. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrid, WCT × RBT hybrids backcrossed to WCT; RBT hybrid, backcrosses to RBT; SD, standard deviation.

Results

We obtained spawning information on 27 WCT, 11 WCT hybrids, 36 RBT hybrids, and 51 RBT from 2000 through 2007 (Tables 1–2). Spawning individuals were found an average of 26 times (standard deviation, SD = 9) during spring (March–June). Radio-tagged fish ranged in total length from 317 to 545 mm (means: WCT = 417 mm, WCT hybrids = 445 mm, RBT hybrids = 409 mm, and RBT = 404 mm) and in weight from 254 to 1527 g (means: WCT = 766 g, WCT hybrids = 805 g, RBT hybrids = 674 g, and RBT = 629 g).

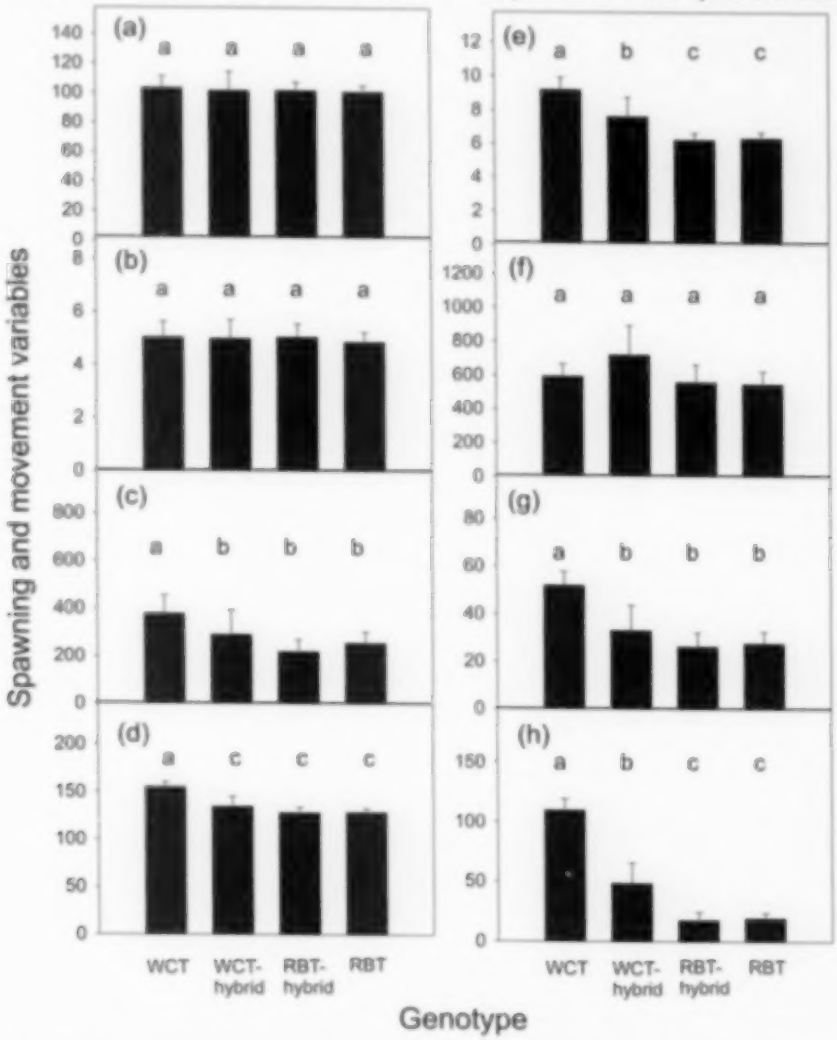
The majority of radio-tagged fish made upriver migrations (98%) and spawned in a total of 29 tributaries to the mainstem, North Fork, and Middle Fork Flathead rivers (Table 1). Only two fish moved downriver to access spawning tributaries to the mainstem. There was no evidence of radio-tagged fish using mainstem or side-channel areas for spawning.

Behavioral differences in spawning and migration

We simultaneously assessed the fixed effects of group, year, and sex on the eight spawning variables using multivariate GLM (Fig. 2). There were significant differences in the spawning and movement variables among groups (MANOVA, Wilks' $\lambda = 0.207$, $F_{[21,213]} = 7.425$, $P < 0.0001$) and years (MANOVA, Wilks' $\lambda = 0.152$, $F_{[42,350]} = 4.119$, $P < 0.0001$), but not between male and female fish (MANOVA, Wilks' $\lambda = 0.837$, $F_{[7,34]} = 2.063$, $P = 0.058$). WCT spawned at higher flows in 2006 and 2007 as compared with 2000 and 2001 (ANOVA, $P < 0.001$), but none of the other seven spawning migration variables was significantly different across years (ANOVA, $P > 0.05$). RBT and RBT hybrids spawned significantly later (ANOVA, $P < 0.001$) and at higher flows (ANOVA, $P < 0.001$) in 2002, likely due to a protracted and higher spring runoff. Additionally, there were no significant interactions among the fixed effects (group, year, and sex) in the overall model. Therefore, we combined the data for all years and genders for subsequent analyses.

MANOVA found overall differences in spawning and migration behaviors among the study groups (Wilks' $\lambda = 0.164$, $F_{[21,330]} = 13.804$, $P < 0.0001$; Table 2; Fig. 2) for five of the eight spawning and migration variables: the flow

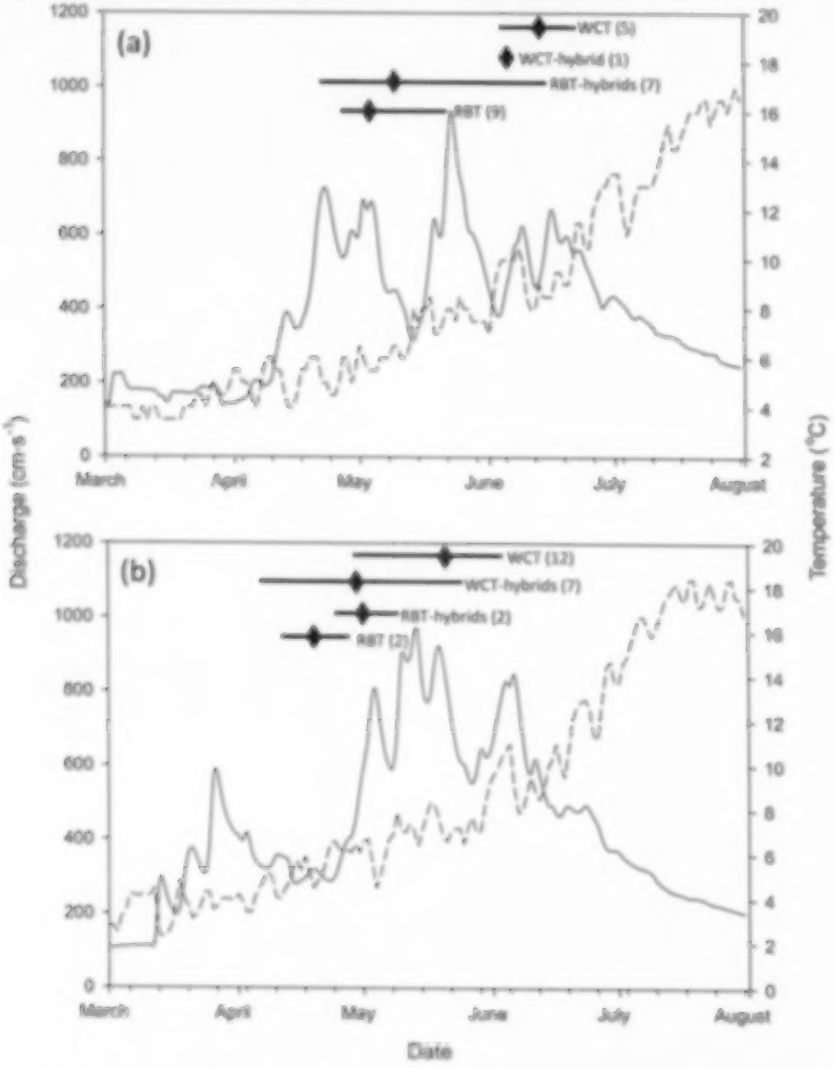
Fig. 2. Comparisons of movement and spawning characteristics (±95% confidence interval, CI) for eight variables: (*a*) beginning migration date (day of year), (*b*) beginning migration temperature (°C), (*c*) beginning migration flow ($m^3 \cdot s^{-1}$), (*d*) spawning date (day of year), (*e*) spawning temperature (°C), (*f*) spawning flow ($m^3 \cdot s^{-1}$), (*g*) migration period (days), and (*h*) migration distance (km). For each measurement, means that do not share letters are significantly different (Tukey's test, $P < 0.05$). Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrid, WCT × RBT hybrid trout backcrossed to WCT; RBT hybrid, WCT × RBT hybrid trout backcrossed to RBT.



at which they began migrating (ANOVA, $F_{[3,121]} = 5.016$, $P = 0.003$), migration period (ANOVA, $F_{[3,121]} = 14.738$, $P < 0.0001$), migration distance (ANOVA, $F_{[3,121]} = 140.329$, $P < 0.0001$), spawning date (ANOVA, $F_{[3,121]} = 21.106$, $P < 0.0001$), and spawning temperature (ANOVA, $F_{[3,121]} = 27.338$, $P < 0.0001$). Tukey's post-hoc comparisons revealed that WCT differed from RBT and RBT hybrids in the flow at which they began migrating ($P < 0.01$), migration period ($P < 0.0001$), migration distance ($P < 0.0001$), spawning date ($P < 0.0001$), and spawning temperature ($P < 0.0001$). Conversely, no differences were found between RBT and RBT hybrids for any of these five variables ($P > 0.05$). Furthermore, there were significant differences between WCT and WCT hybrids for spawning date ($P = 0.001$), migration period ($P = 0.012$), spawning temperature ($P = 0.017$), and migration distance ($P < 0.001$). However, WCT hybrids only differed between RBT and RBT hybrids for migration distance ($P < 0.001$) and RBT hybrids for spawning temperature ($P = 0.036$).

WCT spawned during peak flows and as flows declined following peak spring runoff (May and June) and a mean water temperature of 9 °C (Fig. 3). In contrast, RBT and RBT hybrids spawned during increasing and peak spring flows (April and May) and as temperatures rose to about 6 °C. WCT hybrids spawned throughout spring runoff (April and May) and at intermediate temperatures (mean = 7.6 °C). All groups of fish began migrations on the rising limb of the hydrograph at similar flows and temperatures (Figs. 2*a*–2*c*).

Fig. 3. The spawning periods and mean spawning dates (diamonds) for each taxa as related to the hydrograph and thermograph at Columbia Falls in the mainstem Flathead River in (*a*) 2001 and (*b*) 2007. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrids, WCT × RBT hybrid trout backcrossed to WCT; RBT hybrids, WCT × RBT hybrid trout backcrossed to RBT. Solid lines represent flow and dotted lines represent temperature. Sample sizes (number of fish) are in parentheses.

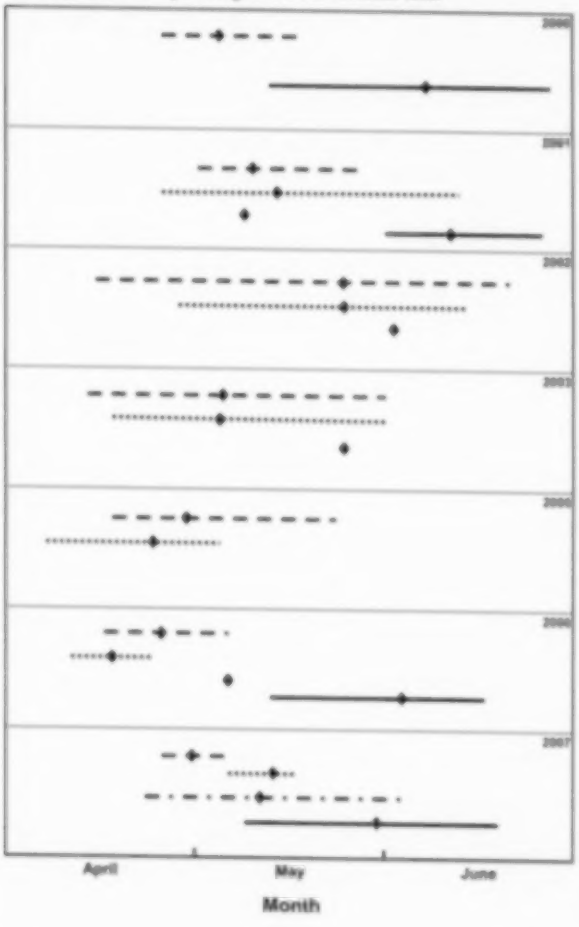


Mean spawning date for WCT (3 June) was significantly later that those of WCT hybrids (13 May), RBT hybrids (5 May), and RBT (7 May) (Figs. 2*d* and 3). Additionally, WCT spawned at significantly warmer temperatures (means: WCT = 9.2 °C, WCT hybrids = 7.6 °C, RBT hybrids = 6.2 °C, and RBT = 6.3 °C; Fig. 2*e*). Migration period for WCT was about twice (mean = 52 days) as long as for RBT and hybrids (means: WCT hybrids = 33 days, RBT hybrids = 26 days, and RBT = 28 days; Fig. 2*g*), likely because WCT migrated significantly farther upriver (mean = 110 km) to access spawning tributaries (Fig. 2*h*) as compared with WCT hybrids (48 km), RBT hybrids (18 km), and RBT (19 km).

Spatial and temporal overlap in spawning

Despite the differences in spawning behavior among the groups of fish, some temporal and spatial overlap occurred (Figs. 3–4). The long spawning periods produced temporal overlap between WCT and hybrid trout in 2001 and 2007 and between WCT and RBT in 2000 (Fig. 4). No temporal overlap was found between WCT and the other groups in 2006, possibly due to small samples sizes that limit the ability to draw inferences that year (N = 9 fish); the observed spawning periods increased with the more fish we tagged. For all years combined, RBT had the longest spawning period (11 March – 20 June) relative to the other groups: RBT hybrids (4 April – 14 June), WCT hybrids (19 April – 3 June), and WCT (9 May – 25 June) (Fig. 4).

Fig. 4. Spawning periods and mean spawning dates (diamonds) of 125 radio-tagged trout during 2000–2003 and 2005–2007: 27 westslope cutthroat trout (solid lines), 11 WCT × RBT hybrids backcrossed to WCT (dashed–dotted lines), 36 WCT × RBT hybrids backcrossed to RBT (dotted lines), and 51 rainbow trout (broken lines). Sample sizes are provided in Table 2. The diamonds with no lines represent the spawning date of individual fish.



Distribution of spawning locations varied geographically among the four groups of fish. WCT spawned in headwater tributaries, RBT and RBT hybrids spawned in the lower drainage, and WCT hybrids generally spawned in the middle portion of the drainage, overlapping with WCT spawning locations (Table 1; Fig. 1). Furthermore, we found evidence that hybridization is spreading upstream via long upstream movements of a few individuals with a high proportion of RBT admixture. Two RBT males made long-distance migrations and spawned in tributaries used by radio-tagged WCT during the study. One RBT migrated 73 km upriver and spawned in the lower section of Hay Creek in 2002, the same general location that a WCT used for spawning in 2000. Similarly, in 2002, a RBT made an upriver movement of 67 km and spawned in Lincoln Creek, the same stream that was used by a WCT and a WCT hybrid for spawning in 2007. Also during the study, we tracked two F_1 hybrids representing previous matings of parental taxa. One F_1 hybrid moved 67 km upriver and spawned in Coal Creek (North Fork) in 2003, which is the same stream that a WCT used for spawning in 2006, and the remaining F_1 hybrid spawned in a tributary near the confluence of the North Fork and mainstem Flathead rivers in 2002, along with several other RBT and RBT hybrids during the study. Finally, individuals with a high proportion of RBT admixture made long-distance movements to spawning areas that WCT hybrids used for spawning; four fish migrated a mean distance of 55 km and spawned in Dutch and Anaconda creeks.

However, the majority of RBT and RBT hybrids spawned in tributaries in the lower portions of the drainage (Table 1; Fig. 1). These streams included (in order of use) Abbot, Rabe, Taylors, Ivy, Third, Dutch, Anaconda, and Mill creeks. Abbot Creek supported a high proportion of spawning by RBT and RBT hybrids (52%), indicating that this location is the main source of introgression in the study area (Table 1). The spawning behaviors of several radio-tagged RBT and RBT hybrids suggest that a former private RBT hatchery (Sekokini Springs) that ceased operations in 1997 (see Discussion), located about 2 km downstream of the confluence of the Middle and North Forks near Abbot Creek, is likely the original source of the recent proliferation of hybridization in the upper Flathead River system. Three RBT and two RBT hybrids made pronounced upstream migrations to the upper mainstem Flathead River and then displayed short upriver and downriver movements in close proximity to the hatchery site, suggesting that they originated from the hatchery, before apparently spawning in Rabe Creek. Moreover, the majority of RBT and RBT hybrids spawned in streams in close proximity (±5 km) to the hatchery during the study.

Discussion

Conservation of native biota requires understanding the invasion patterns and the mechanisms that promote extinction by hybridization. In North America, many populations of native cutthroat trout are threatened by introgressive hybridization with nonnative salmonids, yet prior to our study, little was known about the reproductive factors influencing the spread of hybridization in the natural environment. Our data illustrate that contact between previously allopatric species results in hybridization and introgression due to incompletely evolved reproductive isolation mechanisms. The timing and location of spawning varied spatially and temporally producing zones of contact (e.g., hybrids zones; Barton and Hewitt 1985) between parental species, which results in loss of integrity of the native cutthroat trout gene pool. The spawning distributions and movement patterns indicate that hybridization is being promulgated upstream via long-distance movements of individuals with high amounts of RBT admixture (the continent–island invasion model; Arnold 1997). Additionally, the spatial distribution of later generation backcrosses suggests that hybridization is also spreading by dispersal between neighboring populations (the stepping-stone invasion model; Kimura and Weiss 1964). Combined, these data suggest that hybrid source populations are contributing to the spread of hybridization

in the system and provide evidence that hybridization increases the likelihood of reproductive overlap in time and space, promoting extinction by introgression (Rhymer and Simberloff 1996).

Spatial and temporal distribution of spawning

Based on the spatial distribution of spawning, we found that most RBT and RBT hybrids spawned in tributaries in lower elevations, nonhybridized WCT spawned in the headwater reaches, and WCT hybrids were distributed intermediately in the drainage. However, the spawning distributions also revealed that WCT experience spatial and temporal overlap in spawning with RBT and individuals with various RBT ancestries, mostly in the lower and middle portions of the drainage. These results are consistent with many genetic studies that have been conducted in situations in which RBT are introduced into waters containing native cutthroat trout (Gunnell et al. 2008; Metcalf et al. 2008) and WCT (Weigel et al. 2003; Rubidge and Taylor 2004; Ostberg and Rodriguez 2006) populations. Our telemetry results also corroborate recent genetic studies in the system that found gametic disequilibrium at some of the spawning sites and a decrease in the proportion of RBT admixture with increasing distance from lower elevation tributaries (Hitt et al. 2003; Boyer et al. 2008). Similarly, Henderson et al. (2000) found that the majority of spawning Yellowstone cutthroat trout demonstrated spatial and temporal overlap with RBT and hybrids in lower portions of the South Fork Snake River in Idaho.

The observed spatial distribution of hybrids and parental species appears attributable to upriver invasion of individuals with high amounts of RBT admixture into nonhybridized WCT populations. The purported illegal release of an estimated 70 000 RBT individuals in 1997 from a private hatchery in the lower portion of the drainage likely played a significant role in the recent proliferation of introgression in the system. The vast majority of radio-tagged RBT and RBT hybrids spawned in a relatively few streams located near the probable release point, and some of these individuals appeared to be returning to the hatchery to spawn in the early spring. The observed gradient of hybridization and spawning distributions, however, may not simply be the result of the initial release location, as RBT typically use low-elevation areas of systems in which they are introduced (Fausch et al. 2001), possibly because RBT have higher thermal tolerances (Bear et al. 2007), greater metabolic and growth requirements (Robinson 2007), better swimming abilities (Seiler and Keeley 2007), and high variation in habitat use (Raleigh et al. 1984). For instance, in the east slopes of the Rocky Mountains where RBT have been stocked extensively over a wide range of elevations, Paul and Post (2001) found that RBT are most common at lower elevations where they often compete for food resources and hybridize with native salmonid populations.

We did not find any evidence of radio-tagged fish using mainstems or side-channel habitats for spawning. It is possible that these habitats are not suitable for spawning in this system due to high and sporadic water velocities that wash away fry (Fausch et al. 2001), reduce emergence success (Weaver and Fraley 1993), and provide suboptimal nursery habitat during the first year of growth (Shepard et al. 1984). In contrast, in the South Fork Snake River in Idaho, Henderson et al. (2000) found complete spatial and temporal overlap between introduced RBT and native Yellowstone cutthroat trout within side channels in the mainstem. Also, DeRito (2004) reported that RBT spawned in side channels to a greater extent than Yellowstone cutthroat trout in the Yellowstone River, Montana, but that spatial overlap occurred in one side channel used by the parental taxa and hybrids. However, unlike our findings, DeRito (2004) found minimal evidence of temporal overlap in tributaries used for spawning.

Radiotelemetry was useful for identifying streams in which hybridization is occurring and for understanding how hybridization is spreading in this large, interconnected system. We found that most RBT and RBT hybrids spawned in streams in the lower drainage and that Abbot Creek, which contains a hybrid swarm with a 92% RBT admixture, likely serves as a major source of RBT introgression in the upper Flathead River system. These results are consistent with recent genetic studies in this system (Boyer et al. 2008) and nearby Kootenay River system (Rubidge and Taylor 2005) that found a significant negative correlation in the proportion of RBT admixture with upstream distance from hybrid sources.

Spawning behavior

Our study also provides evidence that individuals with low and high levels of RBT admixture differ in spawning behavior compared with nonhybridized WCT. Our findings indicate that (*i*) WCT display significantly different spawning behaviors than RBT and hybrids, (*ii*) F_1 hybrids and individuals backcrossed to WCT display spawning and movement characteristics generally intermediate to those of WCT and RBT and RBT hybrids, and (*iii*) hybrids backcrossed to RBT behave similarly to RBT. The spawning behaviors of native WCT probably represent adaptations to the environment, which have been selected for at least since the Wisconsin glacial period (~14 000 years ago). WCT may possibly migrate during high spring flows to access the full extent of available habitat and spawn as flows decline to minimize sediment deposition (Weaver and Fraley 1993) and substrate scour (Fausch et al. 2001) and to avoid high water velocities that wash away trout fry (Fausch et al. 2001). Our data suggest that hybridization may produce earlier spawning times, leading to earlier emergence times, and possibly reduced recruitment success. Loss of local adaptations may be especially detrimental to indigenous species during periods of extreme environmental conditions, such as fire, drought, and winter flooding (Leary et al. 1995; Allendorf et al. 2004).

Increased straying rates in the invasive taxon can contribute to extinction of native species by hybridization. Native WCT populations exhibit a high degree of genetic divergence over short geographical distances (Allendorf and Leary 1988; Boyer et al. 2008), indicating low straying rates among populations. Conversely, the rapid spread of hybridization indicates a high rate of dispersal and gene flow from hybrid and RBT sources. Our data indicate that hybridization is spreading upriver, in part, by long-distance movements of individuals with high amounts of RBT admixture that likely strayed into streams containing nonhybridized WCT, which corroborates conclusions from a recent genetics study in the system (Boyer et al. 2008).

Hybridization and reproductive segregation may be dynamic events that vary temporally due to abiotic factors, such as river discharge and temperature, and in many systems, hybridization has rapidly increased over short time frames (Hitt et al. 2003; Rubidge and Taylor 2004). For example, temporal genetic analyses in the Flathead system revealed that hybridization was detected at 57% of the sites sampled from 1998 to 2001, and new RBT introgression was documented in 7 of 11 (73%) sites that were determined to be nonhybridized in 1984. In 2002, a high flow year, we found that RBT and RBT hybrids spawned significantly later than in all other years, and coincidentally or not, we observed long-distance spawning movements by two RBT males into streams containing nonhybridized WCT, facilitating interbreeding among parental types (Muhlfeld et al. 2009*a*). Similarly, DeRito (2004) found evidence of temporal variation in spawning among years in the Yellowstone River system; RBT and hybrids spawned earlier with earlier peak spring runoff, increasing the likelihood of overlap with later-spawning Yellowstone cutthroat trout.

Limitations

Differences in spawning characteristics of cutthroat trout and hybrids may also be attributed to the current spatial distribution of hybridization in the system. Prior to the introduction of RBT in the system, WCT likely used spawning tributaries in lower elevations presently dominated by RBT and hybrids. Therefore, it is plausible that some of the observed differences in spawning behavior (e.g., migration distance and migration period) may be due, in part, to the fact that WCT now persist predominately in higher-elevation tributaries. Additionally, we were unable to measure water temperature and flow within spawning tributaries, and these environmental cues may have differed from the measured variables in the mainstem. Nonetheless, the major differences in spawning behavior appear to be related to temperature and flow, as WCT consistently spawned in streams as flows declined following peak runoff and as stream temperatures approached 9 °C, which is consistent with other studies (Bjornn and Mallet 1964; Shepard et al. 1984; Schmetterling 2001). Photoperiod may also be an important factor linked to spawning time, as recent research has identified a gene in RBT that is linked to a spawning time quantitative trait locus (see Leder et al. (2006) and references therein). This could explain, in part, why spawning date is significantly different between WCT and individuals with some amount of RBT admixture.

The purpose of the hybrid index classification was to describe the multilocus phenotype or genotype of individuals. Because of the random reshuffling of alleles during sexual reproduction, the power to discriminate between parental types and later generation hybrids is a function of the number of marker loci used (Boecklen and Howard 1997). With 13 diagnostic nuclear PINE or indel–microsatellite loci, there is much less than a 1% chance that a first-generation backcross would be misidentified as an F_1. The ability to distinguish between nonhybridized parental species and later-generation backcrosses, however, is more problematic. For example, if an individual fish has 10% of its genes from RBT, there is a 6% chance that it would possess no RBT alleles at the 13 diagnostic indel–microsatellite loci. In contrast, with the six PINE diagnostic loci, the presence of the fragment being characteristic of RBT, there is a 28% chance that no RBT alleles would be detected in such an individual. Consequently, because of this sampling error, we probably overestimated the number of pure parental types and underestimated the number of hybrids. This may explain the fact that the RBT and RBT hybrids differed little in spawning characteristics.

Conservation implications

Although nonhybridized WCT populations still exist, our data indicate that many of these populations are at high risk of genetic introgression. The spatial and temporal differences in spawning between WCT and RBT and hybrids are not absolute and, thus, are likely not strong enough to preclude further hybridization. Environmental factors may also not be strong enough to constrain invasion and subsequent introgression in this system and others (Hitt et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009*b*). Moreover, when reproductive overlap occurs and hybrid progeny are produced, hybridization may spread and genomic extinction of parental taxa can occur, even when hybrid offspring experience reduced fitness due to the "ratchet effect" whereby all progeny of hybrids will be hybrids (Epifanio and Philipp 2001; Allendorf et al. 2004). Muhlfeld et al. (2009*a*) found that small amounts of hybridization markedly reduced the reproductive success of native WCT in a recently invaded stream (Langford Creek) in the Flathead system. Hybridization appears to be spreading, however, due to relatively high reproductive success of F_1 hybrids and high reproductive success of a few males with high levels of admixture, which corroborates our telemetry data.

Currently, there are no official policy guidelines for treating hybrids under the Endangered Species Act (ESA; Allendorf et al. 2004, 2005). Recent debate has focused on establishing a clear definition on what constitutes a "pure" species and what level of anthropogenic introgression is acceptable to maintain the evolutionary legacy of this subspecies (Allendorf et al. 2005; Campton and Kaeding 2005). This study provides insight into this ongoing debate by linking genetic status with behavioral characteristics of nonhybridized WCT and hybrid fish during spawning. Our data revealed that hybrids backcrossed to WCT and RBT differed in spawning behavior compared with nonhybridized WCT, suggesting that hybridization may change local adaptations in native WCT populations by producing earlier spawning times.

Currently, hybridizing taxa are not protected under the ESA and their consideration has been determined on a case-by-case basis. Populations with up to 20% nonnative genetic admixture were classified as WCT under the unit considered for listing in a recent status review (US Fish and Wildlife Service (USFWS) 2003). A primary concern with protecting hybrid populations is that inclusion of these populations as WCT may threaten the persistence of this subspecies by protecting hybrid sources and allowing further introgression and loss of ecological adaptations (Allendorf et al. 2004). Our results suggest that introgressive hybridization erodes discrete reproductive behavior of the native taxon and that hybridization is spreading from source hybrid populations in lower portions of the system. Eradication or suppression of

hybrid populations, therefore, may be required as an effective management strategy for reducing the spread of hybridization. Proactive conservation measures, such as removing adults during spawning and installing barriers to migration (Fausch et al. 2006), are being implemented in the Flathead and other systems to slow or stop the spread of hybridization. Alternatively, in situations where there are still parental individuals present, a selective removal program may be an effective option to constrain or prevent introgression. The ability to accurately visually determine the identity of nonhybridized and hybrid fish is necessary for this approach to be successful. Our results suggest that morphological phenotypic characteristics may provide a reasonably accurate and cost-effective means of identifying adult hybrids with high proportions of RBT admixture in the field; however, morphological identification of individuals with low to moderate amounts of admixture can be unreliable (Leary et al. 1984; Behnke 1992; Weigel et al. 2002). Prior to using field identification, we recommend that biologists first compare the accuracy of the characteristics that they have chosen with genetic identification of individuals with varying levels of admixture (e.g., Weigel et al. 2002).

Genetic studies in this system (Hitt et al. 2003; Boyer et al. 2008) and others (Weigel et al. 2003; Rubidge and Taylor 2005) have concluded that in the absence of physical migration barriers, continued introgression with introduced trout will likely lead to the genomic extinction of WCT. Our telemetry results provide additional support for this prediction. Our study demonstrates that introductions of RBT into previously allopatric populations of WCT may result in genetically admixed populations because of a breakdown in reproductive segregation in time and space, promoting extinction by introgression. We conclude that introgression is likely to continue to spread unless populations with high amounts of nonnative genetic admixture are reduced or eliminated.

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Appendix A

Table A1. Paired interspersed nuclear element polymerase chain reaction (PINE PCR) fragments used for the detection of *Oncorhynchus mykiss* introgression.[a,b]

Taxon	PINE fragment number	No. of base pairs	Primer pair	Primer pair type
O. mykiss	1	70	Hpa1 5′/Hpa1 3′	SINEs
	2	66	Hpa1 5′/Hpa1 3′	SINEs
	3	369	Fok1 5′/Tc1	SINE and transposon
	4	230	Fok1 5′/Tc1	SINE and transposon
	5	395	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment
	6	266	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment
O. clarkii lewisi	1	153	Hpa1 5′/Hpa1 3′	SINEs
	2	72	Hpa1 5′/Hpa1 3′	SINEs
	3	69	Hpa1 5′/Hpa1 3′	SINEs

Table A1 (*concluded*).

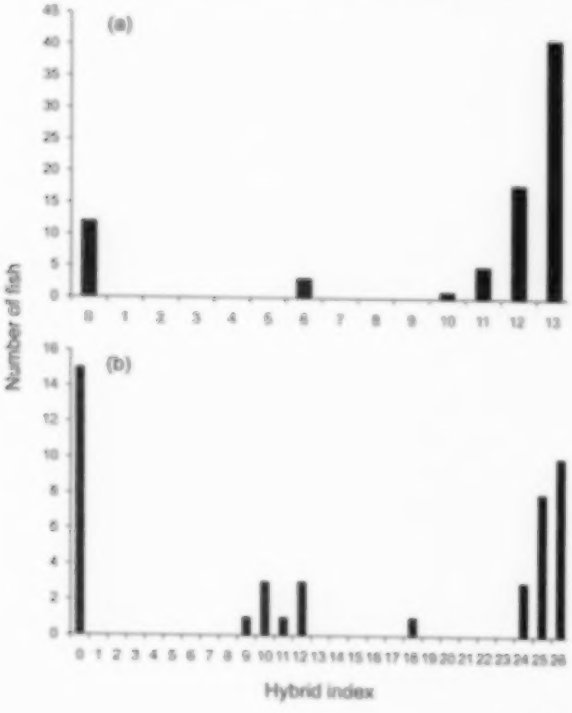
Taxon	PINE fragment number	No. of base pairs	Primer pair	Primer pair type
	4	366	Fok1 5′/Tc1	SINE and transposon
	5	110	Fok1 5′/Tc1	SINE and transposon
	6	388	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment
	7	148	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment

Note: SINE, small interspersed nuclear elements.

[a]Kanda et al. 2002. Trans. Am. Fish. Soc. **131**(2): 312–319. doi:10.1577/1548-8659(2002)131<0312: MGMIHB>2.0.CO;2.

[b]Spruell et al. 2001. Copeia, 2001(4): 1093–1099. doi:10.1643/0045-8511(2001)001[1093:DOHBBT]2.0.CO;2.

Fig. A1. Hybrid index scores of 125 radio-tagged westslope cutthroat trout, rainbow trout, and hybrids based on paired interspersed nuclear element polymerase chain reaction (PINE PCR) of (*a*) 13 dominant nDNA fragments (N = 80 fish) and (*b*) a combination of six co-dominant indel loci and seven co-dominant microsatellite loci (N = 45 fish). The hybrid index score is calculated as the number of nonnative rainbow trout alleles in each fish.



(Table A2 appears on the next page.)

Table A2. Pearson's product moment correlations for eight spawning habitat variables.

		Spawning variables							
Correlations		Beginning migration date	Beginning temperature	Beginning flow	Spawning date	Migration period	Spawning temperature	Spawning flow	Migration distance
Beginning migration date	Pearson's correlation	1	0.7280	0.5625	0.4027	–0.4818	0.3008	0.2092	0.0837
	Significance (two-tailed)		0.0000	0.0000	0.0000	0.0000	0.0007	0.0192	0.3531
	N	125	125	125	125	125	125	125	125
Beginning temperature	Pearson's correlation	0.7280	1	0.2167	0.1666	–0.4719	0.2173	–0.0237	0.0720
	Significance (two-tailed)	0.0000		0.0152	0.0633	0.0000	0.0149	0.7930	0.4248
	N	125	125	125	125	125	125	125	125
Beginning flow	Pearson's correlation	0.5625	0.2167	1	0.3535	–0.1495	0.2536	0.3339	0.2967
	Significance (two-tailed)	0.0000	0.0152		0.0001	0.0960	0.0043	0.0001	0.0008
	N	125	125	125	125	125	125	125	125
Spawning date	Pearson's correlation	0.4027	0.1666	0.3535	1	0.6080	0.7242	0.4730	0.6006
	Significance (two-tailed)	0.0000	0.0633	0.0001		0.0000	0.0000	0.0000	0.0000
	N	125	125	125	125	125	125	125	125
Migration period	Pearson's correlation	–0.4818	–0.4719	–0.1495	0.6080	1	0.4323	0.2713	0.5023
	Significance (two-tailed)	0.0000	0.0000	0.0960	0.0000		0.0000	0.0022	0.0000
	N	125	125	125	125	125	125	125	125
Spawning temperature	Pearson's correlation	0.3008	0.2173	0.2536	0.7242	0.4323	1	0.0189	0.6346
	Significance (two-tailed)	0.0007	0.0149	0.0043	0.0000	0.0000		0.8345	0.0000
	N	125	125	125	125	125	125	125	125
Spawning flow	Pearson's correlation	0.2092	–0.0237	0.3339	0.4730	0.2713	0.0189	1	0.1086
	Significance (two-tailed)	0.0192	0.7930	0.0001	0.0000	0.0022	0.8345		0.2278
	N	125	125	125	125	125	125	125	125
Migration distance	Pearson's correlation	0.0837	0.0720	0.2967	0.6006	0.5023	0.6346	0.1086	1
	Significance (two-tailed)	0.3531	0.4248	0.0008	0.0000	0.0000	0.0000	0.2278	
	N	125	125	125	125	125	125	125	125

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How reliable are the abundance indices derived from commercial catch–effort standardization?

Yimin Ye and Darren Dennis

Abstract: Commercial catch-per-unit-effort (CPUE) data are often standardized to construct indices of stock abundance. The value of such standardization lies in the improvement in the proportionality between the derived index and true abundance. Using the Torres Strait rock lobster (*Panulirus ornatus*) fishery in Australia as an example, we first standardized the commercial CPUE data using a generalized linear model (GLM) and then fitted observation error models to the resulting abundance indices and independent abundance data (as estimated by research diver surveys) to examine the proportionality. While the GLM standardization greatly improved proportionality in comparison with the nonstandardized commercial catch rates, it could produce biased results if the model did not explicitly incorporate variables that had caused changes in fishing efficiency. As most catch–effort standardizations do not model the fishing power component simultaneously, this result may serve as a warning to the potential bias in stock abundance indices extracted from GLMs that are underfitted.

Résumé : On standardise souvent les captures commerciales par unité d'effort (CPUE) afin d'obtenir des indices d'abondance des stocks. L'intérêt d'une telle standardisation consiste en l'amélioration de la proportionnalité entre l'indice obtenu et la véritable abondance. En nous servant de l'exemple de la pêche commerciale de la langouste ornée (*Panulirus ornatus*) du détroit de Torres en Australie, nous avons d'abord standardisé les données de CPUE de la pêche commerciale à l'aide d'un modèle linéaire généralisé (GLM) et nous avons ensuite ajusté les modèles d'erreurs d'observation aux indices d'abondance obtenus ainsi qu'à des données d'abondance indépendantes (estimées par des inventaires de recherche par des plongeurs) afin d'évaluer la proportionnalité. Bien que la standardisation par GLM améliore considérablement la proportionnalité par rapport aux taux de capture commerciale non standardisés, elle peut produire des résultats erronés si le modèle n'incorpore pas de façon explicite les variables responsables des changements de l'efficacité de la pêche. Puisque la plupart des standardisations de la capture–effort ne modélisent pas en même temps la composante de la puissance de la pêche, nos résultats peuvent servir d'avertissement de l'existence d'une erreur potentielle dans les indices d'abondance des stocks obtenus par des GLM sous-ajustés.

[Traduit par la Rédaction]

Introduction

Catch and effort information from commercial or recreational fishers is the most common data source for fisheries studies, as it is cheaper to collect and has greater coverage in space and time than fishery-independent surveys (National Research Council 2000). Besides being used to fit various mathematical models, catch rates most commonly calculated as catch per unit effort (CPUE) are often used as indices of abundance. The use of CPUE data in a fisheries setting dates back to Baranov (1918), with further development in the mid 1950s along with various methods of standardization (Gulland 1956; Beverton and Holt 1957). CPUE data has become more common in modern stock assessments as the primary source of abundance information for many of the world's most valuable and vulnerable commercially and recreationally fished species (Maunder and Punt 2004).

CPUE information should be used cautiously, however, because CPUE may not be an accurate index of abundance (National Research Council 2000). Cooke and Beddington (1984) discussed various scenarios in which CPUE is unlikely to be proportional to abundance and highlighted the possibility of hyperstability, whereby catch rates decline more slowly than actual abundance. Based on a meta-analysis of 297 pairs of CPUE and fishery-independent abundance data, Harley et al. (2001) found strong evidence that commercial CPUE was hyperstable. However, it is also possible to see hyperdepletion in CPUE (e.g., Price and Hilborn 1998). Reasons why CPUE might not be proportional to abundance include the following: (*i*) the relationship between CPUE and abundance may not be linear, as commercial fishing is not a random activity but an operation with clear targets (Harley et al. 2001); (*ii*) in a fishery of mixed vessels and gear types, variation in fishing power among vessels will create varia-

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tions in CPUE unrelated to abundance (Battaile and Quinn 2004); and (*iii*) improvement in fishing technology and accumulation of information and knowledge related to fish distribution and fishing operation will increase fishing power and create hyperstable CPUE (Bishop et al. 2004).

Improper use of CPUE data will result in bias in the assessment of stock status (Maunder and Punt 2004) and, in extreme cases, can contribute to the demise of fisheries when assumptions are not met adequately (Rose and Kulka 1999). It is, therefore, critically important to standardize CPUE so that CPUE is comparable among years and can be used as a reliable index of abundance. Standardization can be a simple process involving the correction of fishing power of the whole fleet through comparison with a standard vessel (e.g., Beverton and Holt 1957). The most recent methods for standardizing catch and effort data compare CPUE data from multiple sources by accounting for various factor effects through the use of mathematical models such as generalized linear models (GLMs), generalized additive models (GAMs), and generalized linear mixed models (GLMMs) (Quinn and Deriso 1999; Battaile and Quinn 2004).

However, even if catch and effort data are standardized to remove the impact of all known factors, there is still no guarantee that the resultant index of abundance is linearly proportional to abundance (Maunder and Punt 2004). For example, unequal and changing spatial distribution of fishing effort can lead to potential bias in the index of stock abundance constructed even through the use of sophisticated models such as GLMs (Campbell 2004). Although such model-based standardization is now a common practice, to our knowledge, no studies have evaluated the reliability of the model-standardized CPUEs. This paper presents a real fishery example of how proportionality between CPUE and stock abundance can be improved using GLM standardization and how changes in fishing power can discount the reliability of the standardized CPUE as an abundance index.

Materials and methods

The data used

To evaluate the reliability of abundance indices calculated through standardization of commercial catch and effort data, we required fishery-independent estimates of stock abundance from the same fishery, because actual abundances cannot be observed in reality. Both the commercial logbook catch and effort data and fishery-independent survey data were obtained for this purpose from the Torres Strait rock lobster (*Panulirus ornatus*) fishery in Australia since 1994.

Fishery-independent research surveys

Annual fishery-independent surveys of the Torres Strait rock lobster population have been conducted since 1989. The first survey aimed to estimate absolute lobster abundance using a stratified random sampling design and allocation of 271 sampling sites, each with two paired belt transects. Subsequent survey designs were optimized using seabed habitat information gained during the first survey. The study area surveyed, which included the fishing grounds, was divided into six sampling strata based on homogeneity of habitat. A second full-scale survey of the lobster population using 313 sites was completed in 2002. In the intervening years, monitoring surveys of 42–50 sites were conducted to estimate the relative abundance of the lobster population. Prior to 2002, two transects were surveyed at each site, but following 2002 only a single transect was surveyed at each site, and the number of sites was increased to improve spatial coverage (for details see Ye et al. 2005). The 75 sites surveyed in 2006 are shown (Fig. 1).

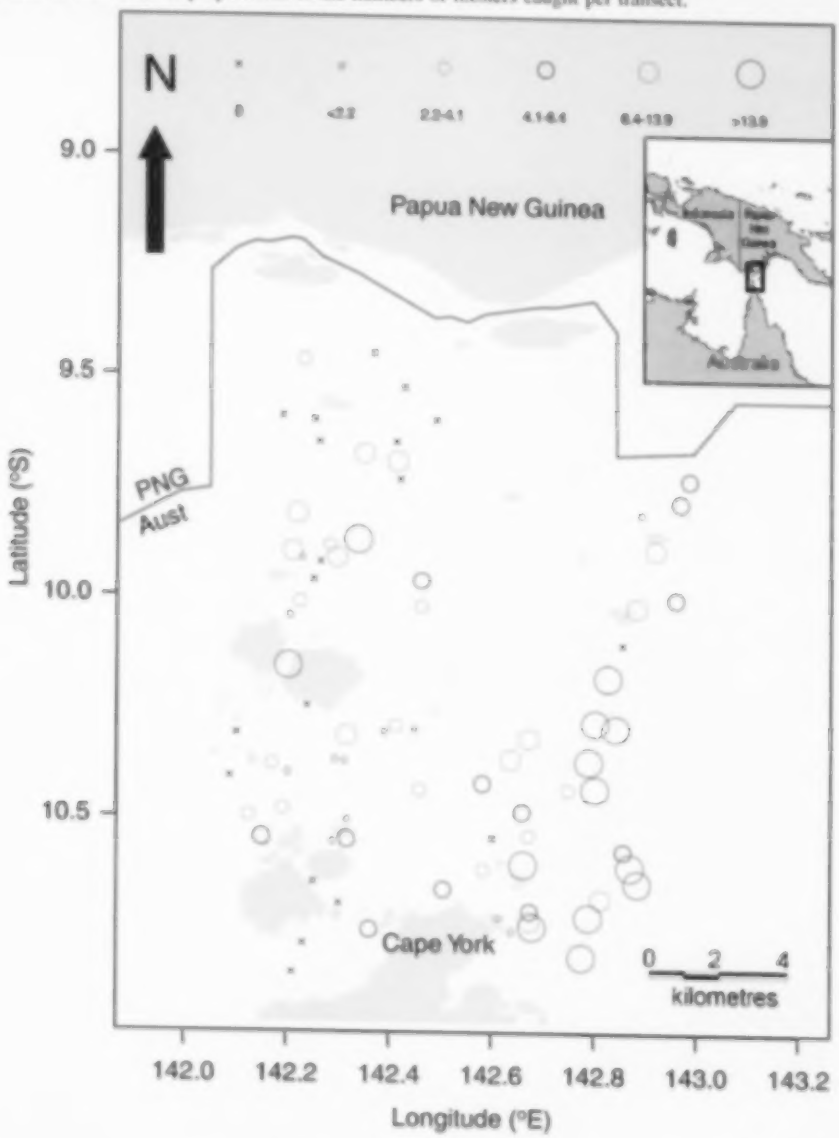
At each site, paired hookah divers swam side by side along a 500 m transect line and recorded the number of lobsters within 1 m on each side of the transect line. The divers speared as many of the lobsters as possible. Hence, a belt transect 500 m × 4 m was surveyed by two divers at each sampling site. In this study, only the 42–50 sites sampled each year were used to construct the fishery-independent abundance indices.

Commercial logbook data

The Torres Strait rock lobster fishery consists of two sectors. One is the freezer vessel sector and the other is the island-based sector. The former sector relies on larger primary vessels (generally 10–20 m in length) with associated tender vessels (generally dinghies <6 m in length), and the lobster divers predominantly employ surface-supplied compressed air equipment (hereafter referred to as hookah diving) to collect lobsters. In contrast, the latter sector relies on smaller vessels, and the lobster divers more commonly employ free diving to collect lobsters. A commercial logbook program was implemented in 1994 to monitor the freezer vessel sector, and the data collected included catch in kilograms, effort, gear type, fishing location, etc. The commercial logbook data used in this study came solely from hookah divers in the freezer vessel sector.

The freezer vessel sector of the lobster fishery consists of about 22 primary vessels, each with 1–5 associated tenders–dinghies. There were 52 350 daily dinghy catch and effort records in the database. To ensure the quality of the catch and effort data used and the technical feasibility of the modelling, a selection process was implemented based on the following criteria: (*i*) Only catches taken by hookah diving were selected. Hookah diving is the predominant fishing method used in the freezer vessel sector, and only about 4% of the total records were clearly identified as catches taken by free diving. There were a further 23% of the records in the database that had no information about fishing gear, and these and the free diving records were excluded. (*ii*) Only data recorded between February and September were selected. Data recorded between October and January were excluded because hookah diving was banned during October to November prior to 2002, and this ban was extended to cover October to January in 2002. In any case, catches taken between December and January were always very small, and this data would not have contributed substantially to this analysis. (*iii*) Data from vessels that had operated in the fishery for less than 5 years or had an annual average catch of less than 5 tonnes were excluded. This exclusion was important because the model used in this standardization includes separate factors for each vessel. Including data for all vessels would have lead to an unnecessarily overparameterized model (Punt et al. 2000).

Fig. 1. Map of Torres Strait, Australia, showing locations of sites sampled during the annual rock lobster (*Panulirus ornatus*) population survey in 2006. The area of the circles is proportional to the numbers of lobsters caught per transect.



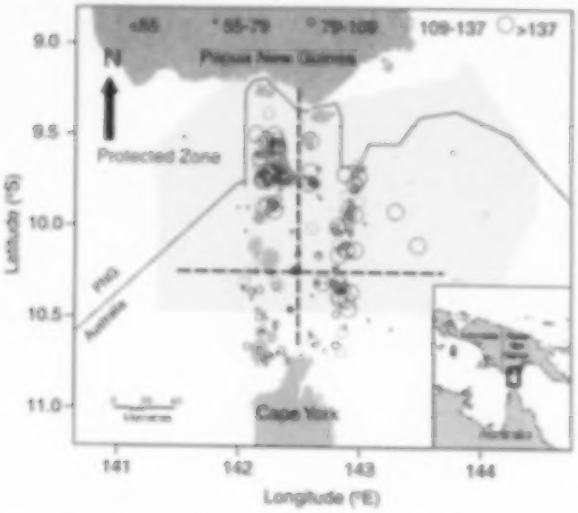
About 65% of the logbook records survived the selection process and were used in this study.

The Torres Strait rock lobster is a migratory species. They migrate out of Torres Strait to breeding grounds in September–October, and the larvae are thought to be transported via currents back to Torres Strait during December to May each year. No large-scale movements have been recorded during their 2.5-year stay in Torres Strait. However, the stock is not evenly distributed across Torres Strait, and as a result fishing effort is not evenly distributed (Fig. 2). Higher catch rates were recorded in the northwest and northeast areas in most years, and as a result fishing effort was generally lower in the southern areas. To account for this difference in the spatial distribution of effort, we divided the whole study area into four quadrants (Fig. 2) and included a region factor in the GLM model that was used to account for regional differences in CPUE and help standardize the commercial CPUE.

Simulation data

To investigate the impact of fishing power creep on the GLM-standardized CPUEs, we simulated data based on the actual commercial logbook records. It was simply assumed that the overall fishing power (P) of the fleet has increased by ~40% since 1994 following the hypothetical pattern shown in Fig. 3, and the actual effort data from the rock

Fig. 2. Map of Torres Strait showing catch rates (kg·dinghy^{-1}·day^{-1}) of Torres Strait rock lobster (*Panulirus ornatus*) fishery in 2005 and the division of the whole fishing area into quadrants (represented by the thick broken lines).



lobster fishery was then discounted by the fishing power creep. The variation in fishing power amongst vessels was assumed to follow a normal distribution, i.e., $N(P_y, (0.2P_y)^2)$.

Analytical methods

Abundance indices based on logbook catch–effort data

Calculating the unstandardized logbook CPUE (U_y')

Using the logbook catch and effort data, mean CPUEs (U_y') can be easily calculated for each year

$$(1) \qquad U_y' = \sum_r W_r \frac{1}{VM} \sum_v \sum_m U_{rvmy}$$

where $U_{rvmy} = C_{rvmy}/E_{rvmy}$, with C_{rvmy} and E_{rvmy} being catch and effort, respectively, of vessel (v) in region (r) at month (m) and year (y). W_r is the weight for region (r) with $\Sigma W_r = 1$, and V and M are the total numbers of vessels and months, respectively. If catchability and spatial distribution of the fishery remains constant over time, U_y' will be approximately proportional to stock abundance.

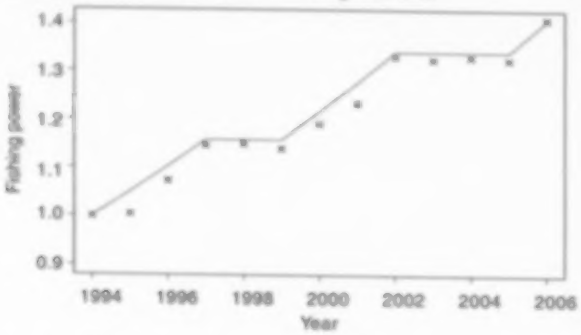
GLM standardization of U_y'

In practice, catchability is highly variable and changes spatially and temporally because of advances in technology, changes in the composition of the fishing fleet, etc. To use catch rates as an index of abundance, the impact on catch rates of changes over time from factors other than abundance must be removed. In this study, a GLM of the following form was fitted to the data to construct abundance indices from catch and effort data

$$(2) \qquad g(U_{rvmy}) = \beta_0 + \sum_p \beta_p X_{prvmy} + \varepsilon$$

where U_{rvmy} is CPUE as in eq. 1, g is a log-link function, β_0 is the global mean for the reference cell, X_p are the p explanatory variables, and ε is normally distributed residual variation. The explanatory variables included in the model are categorical predictor variables: region (r), vessel (v), year (y), month (m), and their interactions. No three-way interactions were considered in this study.

Fig. 3. The pattern of relative fishing power increase of the rock lobster (*Panulirus ornatus*) fleet (solid line) that was used to produce the simulation data and the differences (crosses) between the estimates of catch per unit effort in each year (U_y) from the simulation data and those from the actual logbook data.



The year factor has significant interactions with other factors in the GLM. To develop the index of abundance, interaction terms over factor f were first averaged as follows (Punt et al. 2000):

$$(3) \qquad \bar{\beta}_{xf} = \sum_j W_{f,j} \bar{\beta}_{xf,j}$$

where $W_{f,j}$ is the weighting factor for element j of factor f, with $\Sigma_j W_{f,j} = 1$. In this study, the weighting factors for month and vessel are uniform, but for region, weights were assigned based on each region's area (Fig. 2).

Finally, the abundance index for year y was constructed from the year effect

$$(4) \qquad U_y = e^{\bar{\beta}_0 + \bar{\beta}_y + \sum_f \bar{\beta}_{yf} + 0.5\hat{\sigma}_\varepsilon^2}$$

Note the above formula calculates the abundance index for all years except the first year, which is used as a reference year in the GLM and has the value of $e^{\bar{\beta}_0 + 0.5\hat{\sigma}_\varepsilon^2}$. The antilogarithm of the estimates in the above equation requires a correction factor ($0.5 \times \hat{\sigma}^2$ estimated from residuals) to reduce any bias that may be introduced (Large 1992).

The uncertainty associated with the abundance index derived from the GLM is usually described as confidence intervals. However, when the model has interactions, confidence intervals are not straightforward to estimate. In this study, a random resampling was used to bootstrap confidence intervals of the estimated abundance index. This method treats the predictors as random, potentially changing from sample to sample. Suppose we want to fit a GLM with response variable y and predictors $x_1, x_2, \ldots, x_k$. We have a sample of n observations $z_i = (y_i, x_{i1}, x_{i2}, \ldots, x_{ik})$, $i = 1, \ldots, n$. In random x resampling, we simply select R bootstrap samples from the z_i, fitting the model and save the coeffi-

cients for each bootstrap sample (Fox 2002). We can then construct confidence intervals for the regression coefficients or statistics that are calculated from the regression coefficients, such as U_y in eq. 4.

GLM standardization of simulated logbook CPUE (U'_y)

It has long been recognized that fishing power increases over time because of the introduction of new technologies, evolution in operational and navigational skills, and accumulation of experience. As a result, CPUE will also vary with variables such as physical characteristics of the vessels (e.g., gross tonnages, horsepower, etc), fishing gear (e.g., net design, try gears, etc.), and other equipment (e.g., global positioning system (GPS), echo sounder, etc). Traditionally, fishing power correction was done in the standardization of fishing effort, a process separated from catch rate studies (e.g., Large 1992; Harley et al. 2001). Kimura (1981) was the first to incorporate fishing power modelling into the standardization of commercial catch and effort data as follows:

$$(5) \qquad g(U_{ryqm}) = \beta_0 + \sum_p \beta_p X_{pryqm} + \sum_q \alpha_q Z_{qyv}$$

where g is a log-link function, U denotes CPUE as in eq. 2, X_p are p variables that explain the variation in stock abundance, and Z_q are q variables that influence fishing efficiency. Vessel's engine horsepower, length of net headrope, skipper's experience, use or not of an echo sounder, etc. are often found to be the most important q variables (Bishop et al. 2008) and can be incorporated into eq. 5 in continuous or categorical form.

If eq. 5 represents the real situation, eq. 2 was underfitted to construct the index of lobster abundance. This is the case of model underfit. To investigate the impact of model underfit on the GLM-standardized CPUEs, we fitted eq. 2 to simulation data and then compared the resultant CPUEs (U'_y) with those calculated using the raw data.

Fishery-independent survey abundance index (n_y)

The sample designs of the fishery-independent surveys changed through time to incorporate funding constraints, technology advances, new diving codes of practice, and increased precision. The first population survey conducted in 1989 employed a stratified random sample design, but the subsequent monitoring surveys used a subset of the 1989 sites to estimate relative abundance. In this study, only lobster count data from the common sites used during the monitoring surveys were used. We estimated the mean catch rates (n_y) from each survey and their confidence intervals based on design-based theory of stratified random sampling (Cochran 1977). There was no intention to estimate the absolute population sizes or their uncertainty.

Proportionality between logbook CPUE and stock abundance estimated from fishery-independent surveys

A number of nonlinear models have been proposed to study the relationship between CPUE and stock abundance (e.g., Bannerot and Austin 1983; Richards and Schnute 1986). Following Harley et al. (2001), we used a power curve for its simplicity and flexibility in describing proportionality or disproportionality between CPUE and stock abundance:

$$(6) \qquad I_y = qN_y^b$$

where I_y and N_y are the abundance index and stock abundance, respectively, at year y, and q and b are parameters. When $b = 1$, the model becomes linear, meaning that I is proportional to abundance. If $b < 1$, I declines more slowly than N, which results in hyperstability, and conversely, when $b > 1$, I declines faster than N, which is known as hyperdepletion (Harley et al. 2001).

We do not actually observe the true abundance (N), and it is usually replaced with an estimate n or a relative abundance index (the fishery-independent survey index in this study; Harley et al. 2001). It is clear that the above equation is still valid with an abundance index. However, in this case both dependent and independent variables have observation errors. Ordinary regressions are not appropriate and will result in downward bias in the estimate of b (Quinn and Keough 2002). Therefore, to fit the power curve model, an observation error model is also included for the independent variable used in this study. After replacing N with n, a log transformation of the above equation leads to

$$(7) \qquad \log(I_y) = a + b\log(n_y) + \varepsilon_y$$

where $\varepsilon_y \sim N(0, \sigma_\varepsilon^2)$ is an error term, and a is an intercept parameter. An observation error for the independent variable is incorporated into the model

$$(8) \qquad n_y \propto N_y\, e^{\tau_y}$$

where n_y is observed abundance, and $\tau_y \sim N(0, \sigma_\tau^2)$ is a measurement error. An important assumption used here is the log-normality of the independent variable, so we have $\log(n_y) \sim N(\mu, \sigma_\mu^2)$, which states that the true abundance (N_y) is independently and lognormally distributed (Harley et al. 2001). The measurement error models were fitted using WinBUGS, which avoids the usual constraints on the regression measurement errors and is simple for the Bayesian procedure with hierarchical priors.

The linearly proportional abundance index is achieved when $b = 1$. To investigate the reliability of the GLM-standardized CPUEs, we could simply regress the standardized and nonstandardized CPUEs (U_y and U'_y, respectively, as the observed values of I_y) against the survey indices (n_y) and compare the resultant estimates of b. WinBUGS analysis of covariance (ANCOVA) was used in this study to test the regression coefficients because it allows for error in the x variable. The following model describes relationships between a response variable y and an explanatory variable x, for two groups, indexed by the dummy variable z

$$(9) \qquad y_{ij} = a + b_1 x_{ij} + b_2 z_{ij} + b_3 x_{ij} z_{ij} + \varepsilon_{ij}$$

where $y_{1j} = \log(U'_j)$, $y_{2j} = \log(U_j)$, $x_{ij} = \log(n_j)$, $z_{1j} = 0$, $z_{2j} = 1$, a and b_1 are the intercept and slope, respectively, of the first group of data, and b_2 and b_3 are the difference in intercept and slope of the second group of data in comparison with a and b_1, respectively. As in eq. 7, an observation error is included for the independent variable x. A hierarchical Bayesian method was used to fit the observation error models with WinBUGS (Spiegelhalter et al. 2003).

Results

GLM standardization of commercial CPUE

Fitting the GLM to the commercial lobster catch and effort data showed that the model factors (region, vessel, year, and month) were all statistically significant, as were the interactions (region × vessel, region × year, vessel × year, vessel × month, and year × month; (Table 1). This result showed that catch rate differs amongst vessels and regions and that clear patterns exist for interyear and intermonth variation. The interaction terms suggest that different vessels exhibit different regional patterns and that the annual pattern of catch rate varies from region to region and from vessel to vessel. Although a seasonal pattern of catch rate is well known in this fishery, the results show this pattern differs amongst vessels and years.

Residuals of the log-normal error model were approximately normally distributed (Fig. 4), except for the isolated distribution on the left-hand side, which was caused by zero catches representing <1% of records in the data set. Because of the low number of records of zero catches, we did not use a two-step modelling approach as in Ye et al. (2005), but simply added a small catch (0.1) to these zeros. Overall, the use of a log-normal error model is justified.

Improvement of GLM standardization on the proportionality between logbook CPUE and stock abundance

The annual indices of lobster stock abundance and their bootstrapped uncertainty (one standard error) based on the GLM model fitted to commercial catch and effort data were contrasted with the relative abundance indices estimated from the fishery-independent survey data (Fig. 5). A proportional relationship between the two sets of estimates seems clear, suggesting the standardized CPUEs reflect the variation in real stock abundance quite well.

In general, the uncertainties in the survey-based estimates were larger than those in the model-based estimates, and the uncertainty increased with the value of the abundance index for both estimates. This was particularly evident for the years 1998 and 2005 in which the lobster abundance was the highest recorded based on the survey data.

Fitting the observation error model (eq. 7) to the GLM-standardized CPUEs (U_y) and survey indices gave an estimate of $b = 0.84$ (Table 2). By replacing U_y with U'_y, we estimated $b = 0.58$ (Table 2). Hence, the GLM standardization increased the estimate of b by 0.26, a great improvement in the proportionality between CPUE and stock abundance.

Impact of fishing power creep on the proportionality between logbook CPUE and stock abundance

Indices of lobster stock abundance were also constructed using the same methods but fitting eq. 2 to the simulation data (Fig. 6). It was clear that these indices were higher than those estimated from the actual logbook catch and effort data. By comparing the values of U'_y from the simulation data with those from the actual logbook data, we calculated the percent difference for each year (Fig. 3). The differences were almost the same as the changes in fishing power we used to produce the simulation data. The fishing power component in eq. 5 was not modelled at all in this case, and the model was underfitted. As a result, the model treats the increase in CPUE caused by fishing power creep as an increase in lobster abundance. Fitting the GLM-standardized CPUEs from the simulation data to the power curve model (eq. 7) gives $b = 0.91$, which is 0.07 higher than 0.84 estimated using the actual logbook data (Table 2).

Fitting the ANCOVA model (eq. 9) to the two groups of standardized and nonstandardized CPUEs gives coefficient estimates similar to those from fitting eq. 7 to a single group of data separately. The values for a (3.65) and b_1 (0.56) in Case 4 are close to a (3.64) and b (0.58) in Case 1 (Table 2), and the coefficients calculated from the outputs of the ANCOVA model for the group of GLM-standardized CPUEs are $a + b_2$ (3.56 – 0.75 = 2.81) and $b_1 + b_2$ (0.56 + 0.30 = 0.86) in Case 4, similar to a (2.90) and b (0.84) in Case 2 (Table 2). The parameter estimates for Case 5 can be explained in the same way. The consistency in the estimates of coefficients from the two models suggests the right model was used for the ANCOVA model. Nevertheless, our interest is in b_3, as it represents the difference in the slope of the regression lines between the two data groups. In Case 4, b_3 was estimated at 0.3 with a 95% confidence interval from 0.00 to 0.63 (Table 2), suggesting that the difference is significant and, therefore, the GLM standardization does improve the proportionality between CPUE and stock abundance.

In contrast, in Case 5, $b_3 = 0.05$ and its 95% confidence interval ranges from –0.36 to 0.48 (Table 2). This indicates that the difference in slope between the GLM-standardized CPUEs from the fishery and simulated data is not significant. Therefore, it may be concluded that the overestimation of the standardized CPUEs from an underfitted GLM cannot be detected with the power curve model.

Discussion

In the derivation of the survey and CPUE-based indices, we used a design-based estimator for the survey indices, but a model-based estimator for the CPUE data. Design-based and model-based estimators should provide similar results when the model fits the data well (Stevens 2006; Ghosh et al. 2008). However, design-based estimators should be preferred over model-based ones if an appropriate model cannot be unearthed (Ghosh et al. 2008), because design-based estimators are better for producing unbiased point estimates. The GLMs applied to the survey data had low pseudo-R^2 values from 0.26 to 0.35 (Ye et al. 2005), which is not uncommon in fisheries (Maunder and Punt 2004), suggesting that the models are not precise. Model-based inference may be biased in such circumstances. If both the survey and commercial CPUE data were standardized using GLMs, such biases in both data sets may lead to an over-optimistic conclusion when comparing the two sets of abundance estimates. Therefore, we applied a design-based estimator for the survey data to construct the base abundance indices.

The fishery-independent surveys of the Torres Strait rock lobster (*P. ornatus*) population covered two cohorts: recruiting lobsters aged ~1.5 years old and fished lobsters aged ~2.5 years old. In contrast, the fishery catch consists almost entirely of the latter cohort because of regulations on mini-

Table 1. Analysis of deviance for the generalized linear model (GLM) fitted to the real logbook data of the Torres Strait rock lobster (*Panulirus ornatus*) fishery.

			Residual		
	DF	Deviance	DF	Deviance	*F*
Null hypothesis	27 487	43 262			
Year	12	3 442	27 475	39 820	247.03
Month	7	105	27 468	39 715	12.90
Region	3	807	27 465	38 909	231.67
Vessel	14	2 760	27 451	36 148	169.83
Year × month	83	1 098	27 368	35 050	11.39
Year × region	34	724	27 334	34 326	18.35
Year × vessel	106	2 096	27 228	32 230	17.03
Month × vessel	98	609	27 130	31 621	5.35
Month × region	21	148	27 109	31 473	6.07

Fig. 4. Density histogram of residuals from the generalized linear model (GLM) fitted to commercial rock lobster (*Panulirus ornatus*) catch and effort data for the years 1994–2006.

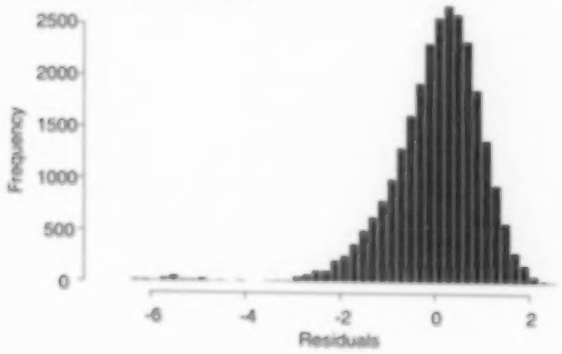
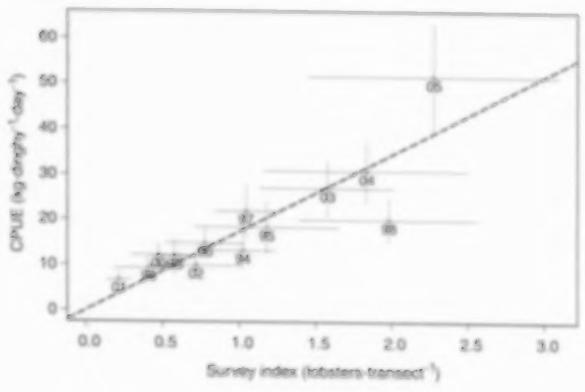


Fig. 5. Plot of the relationship between the fishery-independent, survey-based abundance indices and the generalized linear model (GLM)-standardized catch per unit effort (CPUE) for the Torres Strait rock lobster (*Panulirus ornatus*) fishery. The broken line indicates the proportionality between the CPUE-based and survey-based indices.



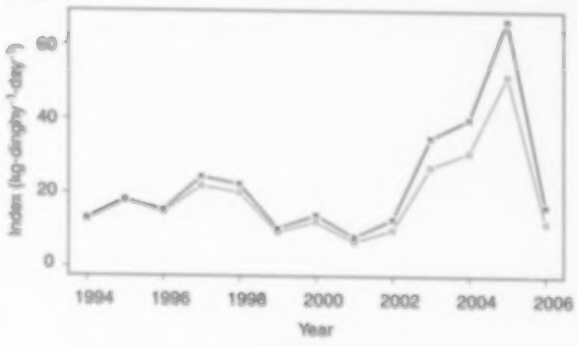
mum legal size and seasonal closures, which effectively protect recruiting lobsters (Ye et al. 2007). Therefore, we only compared the standardized CPUEs with the survey estimates for the 2.5-year-old cohort.

Table 2. Parameter estimates of the observation error model (eq. 7) and the analysis of covariance (ANCOVA) model (eq. 9) used to test the difference between two regressions.

Parameter	Mean	SD	2.50%	Median	97.50%
(1) Raw CPUEs (U'_y)					
a	3.65	0.07	3.52	3.64	3.81
b	**0.58**	**0.17**	0.29	0.56	0.97
(2) GLM-standardized CPUEs (U_y)					
a	2.90	0.09	2.71	2.90	3.08
b	**0.84**	**0.21**	0.52	0.82	1.37
(3) GLM-standardized CPUEs (U'_y) simulation					
a	3.05	0.12	2.85	3.05	3.30
b	**0.91**	**0.29**	0.49	0.88	1.66
(4) Combination of 1 and 2					
a	3.64	0.06	3.52	3.64	3.77
b_1	0.56	0.14	0.31	0.55	0.88
b_2	−0.75	0.10	−0.96	−0.75	−0.54
b_3	0.30	0.16	0.00	0.30	0.63
(5) Combination of 2 and 3					
a	2.89	0.09	2.73	2.89	3.07
b_1	0.85	0.18	0.55	0.83	1.25
b_2	0.16	0.13	−0.10	0.16	0.41
b_3	0.05	0.21	−0.36	0.05	0.48

Note: The following data sets were used: (1) annual average catch per unit effort (CPUE) (U'_y) against survey index ($n_{y,k}$); (2) generalized linear model (GLM)-standardized CPUEs (U_y) against $n_{y,k}$; (3) GLM-standardized CPUEs from the simulation data (U'_y) against $n_{y,k}$; (4) combination of data sets 1 and 2; and (5) combination of data sets 2 and 3. Numerals in bold font highlight the increase in the estimates of *b* when the CPUEs were GLM-standardized and when fishing power increased. SD, standard deviation.

Fig. 6. Torres Strait rock lobster (*Panulirus ornatus*) abundance indices extracted from generalized linear model (GLM) standardization; circles indicate values estimated using the commercial logbook data of the lobster fishery; crosses indicate values estimated using simulation data that were modified from the logbook data by discounting the fishing effort as a result of fishing power creep.



There are two general ways to bootstrap a GLM. One is random *x* resampling or case resampling. The other is called fixed *x* resampling or model-based resampling. In contrast with random *x* resampling, fixed *x* resampling treats the predictor values used in the model as fixed with the replication of the study (i.e., the model matrix **X** is fixed; for details see Fox 2002). The bootstrap coefficient standard errors for fixed *x* resampling are usually smaller than those for random

x resampling because in fixed x resampling residuals are decoupled from the original observations. Consequently, if the model is incorrectly specified — for example, if there is unmodelled nonlinearity, nonconstant error variance, or outliers — these characteristics will not carry over into the resampled data sets (Fox 2002). For this reason, we performed random x resampling in this study. The bootstrap was done using Davison and Hinkley's (1997) boot library in S-PLUS (Insightful Corporation, Seattle, Washington).

The analysis presented here is reliant on the assumption that the fishery-independent survey index is proportional to actual abundance. If the surveys were carried out within some random sampling framework, then one would hope that this assumption would hold (Harley et al. 2001). The first lobster survey in 1989 used a stratified random design, but a subset of these sites was surveyed in subsequent years to estimate relative abundance. Nevertheless, as the survey covers the whole study area and the same sampling sites are visited each year, the proportionality between the survey index and stock abundance should still hold, though standard errors may be underestimated (Van der Meer 1997).

Logbook catch and effort data is not available for the whole Torres Strait rock lobster fishery, and therefore, only data from the freezer vessel sector could be used in this study. The freezer vessel sector contributed up to 80% of the total catch prior to 2001 and 40%–75% of the catch from 2001 to 2006, partially because of the management initiatives to increase the share of the catch for Torres Strait islanders (Ye et al. 2007). Given that most of the catch comes from the freezer vessel sector, the catch and effort data from this sector should reflect actual variation in stock abundance.

The GLM standardization of commercial catch and effort data for the Torres Strait rock lobster fishery increased the estimate of b from 0.56 to 0.84 (Case 4). Although the model standardization is not able to produce indices that are fully proportional to stock abundance ($b = 1$), this example demonstrates that it can make a major improvement in the proportionality of the abundance index in comparison with the nonstandardized CPUE.

The GLM model explained 27% of the overall variation in the logbook catch and effort data. This fraction of explained variation is low by normal statistical standards, but is in the range that most catch–effort standardizations can achieve. For example, standardization used by Anderson (2003) explained 14.5% of the non-zero catch and 7.5% with the probability of a non-zero catch; standardization used by Yamada et al. (2006) explained 17.2%–23.8% of the overall variation. It is worth noting that the GLM was not fitted by stepwise variable selection, but used all the variables available. If more information on fishing activity was available, the model performance would certainly improve. However, despite the low fraction of the overall variation explained by the GLM model, the standardized CPUE was nearly proportional to the survey index with $b = 0.86$. This result suggests that the reliability of catch–effort standardization should not be judged based on the model's capacity to explain data variation.

Confounding between fishing power creep and stock abundance fluctuation is a well-known factor that leads to bias in the estimates of abundance indices. In fitting the GLM to the Torres Strait rock lobster logbook data, no consideration was given to the fishing power component. We believe fishing power creep is occurring in the lobster fishery, but may not be important because (*i*) fishers conduct hookah diving to spear lobsters, and there have not been substantial changes in the capacity of this diving method over the last decade; and (*ii*) the basic operating unit in this fishery is a dinghy, which operates independently of the freezer vessel. The small dinghies have no other equipment used to select diving sites or handle the catch. This has not changed over the last decade. Though some modern technologies have been taken up by the freezer vessels recently such as GPS, depth sounders, and satellite phones, it is unlikely this technology has had a positive impact on the efficiency of dive fishing. Nevertheless, accumulation of knowledge of lobster behaviour and experience in selecting fishing grounds and in the fishing operation might still have a positive impact on fishing efficiency. If this is the case, the estimate of b in eq. 7 may be biased, though not significantly (Case 5). However, this would not change our evaluation of what the GLM standardization can achieve, because we compared the estimate of proportionality parameter (b) from the nonstandardized CPUEs with that from the GLM-standardized values.

Although fishing power creep exists in most fisheries, it was often not explicitly incorporated or considered in catch–effort standardization models (for more examples, see articles in Xiao et al. 2004). This is probably because data on vessel characteristics and other variables that have influence on fishing efficiency are not always available. Our simulation results show that the increase in fishing power will be translated into the increase in abundance in cases where the model is underfitted. Consequently, the standardized CPUEs will overestimate the true abundance. Our results may serve as a warning to the potential bias in the index of abundance extracted from GLMs that do not explicitly model the fishing power component in eq. 5. This kind of bias cannot be detected through the power curve model. Separating fishing power from the indices of abundance might require the impact of vessel characteristics to be estimated separately (Bishop et al. 2004). In the future, greater attention should be paid to the collection of data that can explain the variation in fishing power and to the development of new techniques that can better split the confounding between fishing power and stock abundance.

The simulation conducted in this study was used to demonstrate qualitatively the consequence of ignoring fishing power creep in the construction of an abundance index from commercial catch and effort data. We did not intend to carry out more comprehensive simulations, and conclusions from the simulation study of Chen et al. (1999) apply here. The effect of model underfitting (with some causal variables excluded as covariates) leads to inflated coefficients and underestimated variances of the coefficients. The overestimation of coefficients increases as the correlation coefficient (ρ) between the variable included and the one excluded and the variance (η) of the excluded variables increase. In contrast, the variances of the estimated coefficients decrease with ρ and η.

We used the Torres Strait rock lobster fishery as an example of proportionality between abundance indices derived from fishery-independent surveys and standardized CPUE. We used this data because of the availability of a reasonably long time series of both survey and CPUE data. The fishing method, involving spearing lobsters while hookah diving, is fairly unique to this fishery. However, the general conclusions made here should be applicable to other fisheries provided that the fishery-independent surveys employ the same gear and fishing method as the commercial fishery. Commercial fishing generally develops to maximize the efficiency in finding and capturing fish with the selected gear and method.

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PERSPECTIVE / PERSPECTIVE

Behavioural toxicity of organic chemical contaminants in fish: application to ecological risk assessments (ERAs)

Peter D. Robinson

Abstract: Chemical pollutants rarely attain acutely lethal concentrations in nature; thus the majority of their effects are expected to be sublethal. Estimation of the likelihood of effects from exposures to sublethal concentrations of contaminants in effluent plumes downstream of point sources poses a challenge when conducting ecological risk assessments (ERAs). This is an issue for regulatory agencies worldwide. This paper reviews the importance and availability of information on behavioural toxicity and identifies opportunities for its inclusion in ERAs. One of the major advantages of using data on behavioural effects is that they are more sensitive indicators of potential for impacts on survival in the field than are measures of lethality. Indications from available data for fish suggest that behavioural effects of organic contaminants often occur at concentrations 1 to 2 orders of magnitude lower than those found to elicit mortality. As a result, it is believed that the use of data on behavioural toxicity in ERAs could benefit the assessment process a great deal, allowing for the consideration of more ecologically significant and protective hazard and exposure scenarios.

Résumé : Les polluants chimiques atteignent rarement des concentrations aiguës létales en nature; on s'attend donc à ce que leurs effets soient sublétaux. L'estimation de la possibilité d'effets dus à une exposition à des concentrations sublétales de contaminants dans les panaches d'effluents en aval d'une source ponctuelle représente un défi important dans les évaluations des risques écologiques (« ERAs »). C'est un problème pour les organismes de règlementation à l'échelle de la planète. Ce travail passe en revue l'importance et la disponibilité de l'information sur la toxicité comportementale et signale les occasions de l'inclure dans les ERAs. Un des avantages majeurs de l'utilisation des données sur les effets comportementaux est qu'il s'agit d'indicateurs plus sensibles des impacts potentiels sur la survie en nature que ne le sont les mesures de létalité. Les données disponibles sur les poissons laissent croire que les effets comportementaux des contaminants organiques se manifestent souvent à des concentrations 10 à 100 fois moins élevées que celles qui causent de la mortalité. En conséquence, l'utilisation de données de toxicité comportementale dans les ERAs devrait vraisemblablement améliorer considérablement le processus d'évaluation, permettant la prise en considération de scénarios de risque et d'exposition plus significatifs et plus protecteurs au niveau écologique.

[Traduit par la Rédaction]

Introduction

Behavioural ecotoxicity data have not been widely used by regulatory agencies in the assessment of risks from industrial chemicals, but there exists considerable opportunity for doing so. This paper will attempt to demonstrate how further advances in the incorporation of behavioural effects into ecological risk assessments (ERAs) could be made. The focus of this particular work is on organic compounds, and though it is acknowledged that thermal effluents have a major role to play in behavioural and sublethal effects, particularly with respect to preference and avoidance of effluents (Cherry and Cairns 1982), they will not be considered within the scope of this paper. For the purposes of this paper, a distinction is made between behavioural effects and more traditional sublethal chronic effects generally considered within the scope of a regulatory risk assessment. In the context of the discussion in this paper, "chronic effects" refer only to endpoints such as reproductive success, reductions in growth, and fertility and as such are not within the scope of this paper. Rather, the focus here will be on behav-

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ioural effects observed in aquatic organisms exposed to sublethal concentrations of pollutants, the implications for the life cycle, habitat, species interactions, and survival of affected biota, and how these may be better utilized within the framework of an ERA. It is acknowledged, however, that a behavioural test can indicate changes that may impact on chronic endpoints such as survival, growth, or reproduction (Beitinger 1990).

Why consider behavioural effects in ERAs?

Behavioural effects are important in the assessment of risk from chemical exposures in the environment. Fish, for example, may successfully survive brief exposures to lethal concentrations (e.g., concentrations greater than LC_{50}, i.e., that required to kill 50%) and then through adaptive behaviour, such as avoidance, retreat to more favourable locations in the environment, if they are able to do so. As a result, a disconnect can exist between the endpoints and concentrations measured and generally used within ERAs and those likely representing ecologically "significant" exposures in the environment.

Changes to behaviour from sublethal exposures are considered to be among the most sensitive indicators of environmental disturbances, far more so than "extreme" endpoints such as organism mortality (Gerhardt 2007). Indeed, behavioural effects may be observed at concentrations that are orders of magnitude below those observed to elicit mortality (e.g., LC_{50}) in standard testing (Beitinger 1990; Little and Finger 1990; Little and DeLonay 1996). Compared with endpoints such as survival, behavioural effects have been reported to be often 10 to 100 times more sensitive (Gerhardt 2007).

In addition, behavioural effects represent a less invasive approach to hazard testing, which offers the benefit of being more publicly "acceptable", while also providing more relevant and ecologically significant measures of impacts of contaminants in the environment than acute toxicity testing (Dell'Omo 2002). Sublethal testing offers an opportunity to provide a "nondestructive" means to conduct animal testing, providing quantifiable results, while still being sensitive and ecologically relevant (Grue et al. 2002; Gerhardt 2007). Recent attention focusing on the reduction of the impacts on animals from testing has made the utility of behavioural effects all the more apparent.

It can be argued that sublethal effect testing can be more complex and resource intensive than other types of toxicity testing. However, it has been noted that even acute toxicity tests with fish (e.g., 96 h LC_{50}), which are time and cost efficient relative to many other tests required to evaluate hazard, still represent a considerable strain on the resources of a standard laboratory (Kondaiah and Murty 1994). Also, with advances in technology and computational capacity, the obstacles to conducting behavioural toxicity testing have been reduced considerably (Kane et al. 2005). Even when lethal and more traditional sublethal animal testing are required, authorities should be encouraged to incorporate measures of behavioural effects within testing protocols and observations (Little et al. 1990; Grue et al. 2002).

It is argued that harmful effects at sublethal concentrations occur routinely in standard acute and chronic toxicity testing, but that they are rarely measured, quantified, or recorded in a systematic fashion (Little et al. 1990). Extending observations in toxicity testing to include behavioural effects would enable workers to capture effects at lower test concentrations or at earlier stages within the exposure period, where behavioural and physiologic effects (e.g., reduced swimming capacity, schooling behaviour, etc.) can often be routinely and readily observed. Behavioural effects are frequently noted in the toxicity literature; however, these are often referenced in terms of "abnormal behaviour observed," with information on the nature of the behavioural changes and the concentrations and exposure durations that induced them rarely discussed (Giattina and Garton 1983; Little et al. 1985). Although many behavioural observations have been reported anecdotally in the literature, an effort has been made in this paper to focus on those studies that directly quantify behavioural endpoints.

The importance of sublethal effects in ERAs

It is argued that sublethal effects should be of greater significance than acute and chronic measures of survival because more organisms experience such sublethal exposures to contaminants (Sprague 1971; Scott and Sloman 2004; Kane et al. 2005). Not only are organisms less likely to encounter elevated concentrations of chemicals in nature, but it is also assumed that mobile organisms are generally repulsed and avoid concentrations that are acutely toxic. Steele et al. (1996) argue that sublethal responses such as behavioural bioassays measuring avoidance and preference in fish are very useful as a means of screening pollutants. Although a considerable body of information exists in the area of behavioural effects to fish, there is a considerable gap that has not been bridged between the availability of this information and the use of it in a meaningful and consistent manner. In most cases, ERAs conducted by regulatory agencies (e.g., European Union, US Environmental Protection Agency, Environment Canada) have relied on the determination of effects concentrations from widely accepted, straightforward, and standardized test protocols (e.g., OECD test guidelines) (European Chemicals Bureau (ECB) 2003; US Environmental Protection Agency (US EPA) 1994). This has ultimately led to a reliance on acute endpoints for aquatic organisms such as LC_{50}s–EC_{50}s, or NOECs–LOECs (or EC_{10-20}) for chronic endpoints. When chronic data are not available, however, an extrapolation factor often is applied to available acute data to estimate chronic values.

The use of these acute and chronic data, however, poses a challenge when risk assessors attempt to understand the ecological significance of an effluent plume, or downstream concentration, in a watercourse. The challenge stems from attempting to gain an understanding of what, if any, ecological significance these toxicity values have in estimating the risk to organisms in a receiving watercourse. To further complicate the issue, exposure durations and concentrations may vary considerably within the environment (e.g., in an effluent plume), and organisms also may have the ability to either decrease or increase their exposure based on modifications to their behaviour. Use of data relating to behavioural changes has the advantage over use of classical lethal and sublethal toxicity endpoints in that they reflect both the

direct and indirect effects of contaminants; for example, they may provide evidence that contaminant exposures cause functional deficits in affected organisms such as disruption of metabolic function (Dell'Omo 2002). Contaminants and effluents that cause mortality are certainly of concern from an ERA standpoint. However, generally speaking, most regulatory agencies protect against the release of concentrations causing severe (acute) effects. More often than not, such regulation or control of effluents and pollutants is administered at the end-of-pipe (US EPA 1988; Canadian Council of Ministers of the Environment (CCME) 2005). As a result, within the context of ERAs, it is the occurrence of behavioural effects to aquatic biota that merits greater attention.

Fish represent an ideal class of organisms for behavioural testing, as many ecologically relevant behaviours are readily observed and quantified in a controlled setting (Scott and Sloman 2004). Many fish are also highly mobile organisms, capable of sustained swimming speeds and rapid "burst" movements. Fish also possess sensitive chemoreception capabilities, allowing for the discrimination of chemicals in their environment and, ideally, an observable behavioural response to the presence of the chemicals (Sutterlin 1974; Beitinger and Freeman 1983). In addition, a great deal is known with respect to fish physiology, which makes the task of establishing a link between physiologic changes and corresponding behavioural changes attainable (Scott and Sloman 2004). Behaviour is a result of a variety of biotic and abiotic factors and, as such, represents an animal's response to internal (i.e., physiological) and external (i.e., environmental) factors. As a result, behaviour can be an important means to identify the ways in which fish react and adapt to the presence of contaminants in their environment (Dell'Omo 2002; Gerhardt 2007). Changes in fish behaviour are ideally suited to measuring "stress" as a result of contaminants in their environment (Beitinger and McCauley 1990). Although this stress may represent an adaptive response by fish to environmental changes and may represent a beneficial response in the short term, it also may represent a response that may eventually adversely affect survival at both the individual and population level (Dell'Omo 2002). Even adaptive responses carry a cost to the fish in terms of requiring additional energy to restore homeostasis as a result of chemical stressors (Beitinger and McCauley 1990).

Availability of behavioural toxicity information for ERAs

A number of researchers have reviewed the issue of behavioural toxicity in fish and reflected on the state of the science. Reviews and key papers on the subject include those done by Beitinger (1990), Little and Finger (1990), and Steele et al. (1996). More recently, additional contributions to the field have been made by Dell'Omo (2002), Scott and Sloman (2004), and Gerhardt (2007), among others. To be certain, beyond the aforementioned reviews in the field, there is a considerable amount of research on individual chemicals, or classes of chemicals, waste effluents, test methods, etc., that have been conducted in the area of behavioural toxicity and sublethal effects.

A number of ecologically relevant behavioural endpoints have been measured, including feeding and swimming behaviour, cough rates, changes in activity levels, and intra- and inter-specific social behaviours (Drummond and Carlson 1977; Beitinger 1990; Little and Brewer 2001). Generally speaking, however, the tendency in much of the work conducted is to evaluate avoidance and (or) attraction. This is no doubt due in part to the relative difficulty of measuring other behavioural effects and the lack of standardized protocols (Kondaiah and Murty 1994; Vogl et al. 1999; Kane et al. 2005). As a result, the avoidance and attraction behaviours of fish observed in the presence of contaminants represents an excellent starting point for regulators to begin exploring the role of behavioural toxicity in their ERAs.

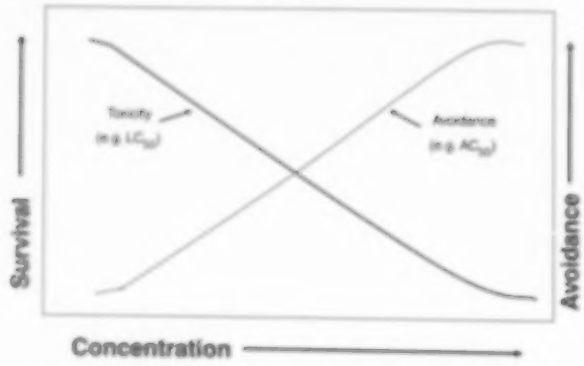
Fish may be unable to avoid high concentrations of contaminants if they produce an immediate physiologic shock or otherwise interfere with the sensory capabilities of the organism (Beitinger 1990). Assuming that the exposure concentration does not interfere with the fish's critical life functions immediately upon encountering the contaminant, avoidance or preference towards the contaminant likely represent one of the first behavioural responses to adverse stimuli (Beitinger and Freeman 1983). It has been observed that fish can rapidly sense and avoid a "hostile" environment, responding within 5 min of exposure (Cherry and Cairns 1982). It is generally recognized that there is an inverse relationship between potential for direct toxicity and avoidance (Gray 1990). This inverse relationship is illustrated in Fig. 1.

A relationship between avoidance and survival shows that as the concentration increases, survival is generally expected to decrease (Fig. 1). However, with avoidance, the opposite is often observed. As concentration increases, survival may also increase, as avoidance responses in fish work to reduce exposure to ever-increasing lethal concentrations of contaminants (Gray 1990). This works to the organism's benefit, at least in the short term, assuming that an alternative suitable habitat exists for the fish to move to, thereby avoiding potentially lethal exposures (Grue et al. 2002).

Though behavioural responses such as avoidance may be more directly induced by irritation to the fish at the gill surface and mucous membranes, scales, etc., the role of chemoreception in avoidance (and preference) of aquatic pollutants is likely of more importance for fish exposed at sublethal concentrations (Little and Brewer 2001). The question arises then, if the chemical is not an irritant to the organism, but rather is detected as an odour that it has not encountered before, will the organism necessarily avoid it (Sutterlin 1974)? Chemoreception represents an important mechanism by which fish first sense, then respond to, the presence of a contaminant in the aquatic environment.

Sutterlin (1974) noted that it is worthwhile to question how an organism can act to avoid a chemical upon first encounter when the chemical is not naturally occurring (i.e., xenobiotics), irrespective of whether or not it is toxic. Undoubtedly, many xenobiotics are not detected or perceived, even though they may well be toxic to the organism. Similarly, there are no doubt chemicals that are perceived but may not be toxic at the concentrations encountered (Sutterlin 1974). If the chemical has been present for some time, or occurs naturally, then it is understandable that avoidance of it may exist as an adapted response (Sutterlin 1974). However, even substances that are not "novel" to the fish may

Fig. 1. Relationship between avoidance and survival with concentration (adapted from Gray 1990, p. 64). AC_{50}, concentration inducing an avoidance of 50%; LC_{50}, concentration inducing a lethality of 50%.



not be sensed because of odour "masking," which may occur when contaminants are present in a mixture such as in industrial effluent or municipal waste water (Smith and Bailey 1990). Similarly, a contaminant may mask odours that migratory fish (e.g., salmon) may use to orient themselves based on an "olfactory memory" and to locate rivers and spawning tributaries (Sutterlin 1974; Little and Brewer 2001). The use by humans of aerosols for the concealment of household and personal odours is evidence enough of the effectiveness of this manipulation of chemoreception. Hence, it is reasonable to assume that such odour masking can also occur and alter behaviour in fish by concealing "normal" chemical cues utilized throughout their life cycle (Sutterlin 1974). Contaminants can also damage chemoreceptors, the effects of which may or may not be reversible (e.g., binding or alteration of chemosensory receptors) (Scott and Sloman 2004).

On the surface, avoidance of aquatic pollutants would appear to be a beneficial response to contaminant exposure. However, avoidance of sublethal levels of toxicants, and indeed pollutants at nontoxic levels, may not be beneficial if in doing so the response restricts the animal from spawning–rearing areas and from feeding and interferes in general with the life cycle and habitat of an organism and (or) population (Sutterlin 1974; Little et al. 1985). As a result, avoidance of contaminants in the aquatic environment can be of considerable ecological importance.

Based on data presented (Table 1), it is clear that many contaminants elicit avoidance behaviour in fish at concentrations considerably lower than those known to elicit a lethal toxic response. Much of the avoidance data available has focused on pesticides, chlorinated compounds, and metals (which have not been explored in this paper). Chlorine is of special interest when examining avoidance in fish. Chlorine, chlorinated compounds, and waste effluents are thoroughly studied with regard to preference and avoidance reactions in fishes, and there is evidence indicating that fish perceive chlorine at very low concentrations (Larrick et al. 1978; Giattina and Garton 1983). Chlorine is considered to be an irritant to fish, causing damage to gill tissue, which may explain the avoidance behaviour of fish towards chlorinated contaminants in the environment (Cherry et al. 1979).

With respect to studies on preference of fish for contaminants, it is interesting to note that many of the preference responses observed are found in studies on contaminated waste streams (Table 2). Waste effluents are generally complex mixtures, making it difficult to distinguish what precisely is responsible for the preference response in fish. Many waste effluents, e.g., municipal wastewater, have been found to attract fish, although whether this occurs as a result of temperature differences between the waste effluent and the receiving watercourse or some attractive chemical component is unclear (Smith and Bailey 1990).

In many cases where preference for a contaminant is noted in fish, it is often concentration-dependant, with both avoidance and preference observed depending on the concentration tested (Folmar 1976; Smith and Bailey 1990). Given the complex nature of the preference response in fish, its usefulness as a sensitive behavioural endpoint for ERAs is probably limited, except in the case of wastewater effluents. This relationship between preference responses and wastewater effluents further complicates interpretation of this form of sublethal behavioural effect because they tend to be complex mixtures, and thus their constituents, and their relative importance in inducing an observed preference response, is generally unknown. Some of the sublethal effects on swimming behaviour observed over a variety of contaminants and species are illustrated (Table 3). Impairment of behavioural functions such as swimming, schooling, feeding, prey capture, and predator detection have been well documented at concentrations far below (by orders of magnitude in many cases) those concentrations known to elicit more traditional acute or chronic toxicity (Giattina and Garton 1983; Beitinger 1990; Little and DeLonay 1996). Adverse effects on a fish's ability and capacity to swim have a number of ecologically significant implications for the survival of the individual and ultimately the affected populations (Little and Brewer 2001). Impacts include the ability of a fish to maintain position relative to the current (rheotaxis) and the ability to maintain swimming velocity and activity, both of which are critical for the successful performance of life functions (Beitinger and McCauley 1990; Little and Brewer 2001).

An interesting variety of other responses in fish to chemical insult is also illustrated (Table 4). These effects have been separated from the effects described in previous tables, even though there may be considerable overlap in some cases; for example, prey capture is likely heavily linked with swimming capacity. Little et al. (1990) also observed that concentrations found to significantly affect the strike frequency and prey capture rates in fish also corresponded to concentrations found to reduce growth in the same species.

Cough rate is another interesting endpoint, as it a relatively easy parameter to measure in a standardized fashion, and fish cough even in clean water, allowing for measurement of differences in cough rates between fish in clean and treated samples (Beitinger and McCauley 1990). A fish cough, or gill purge, produces a quick movement of water that is apparently intended to flush the gills of particulate matter or foreign material. Beitinger and McCauley (1990) in a review of the literature noted that 12 of 18 organic compounds (as well as 11 of 12 inorganic compounds) were found to significantly increase cough frequencies of various

Table 1. Avoidance responses in fish to chemicals.

Pollutant	Sublethal concentration ($mg \cdot L^{-1}$)	Test duration (h)	LC_{50} ($mg \cdot L^{-1}$)	LC_{50} test duration (h)	Fish species	Reference
Polyoxyethylene monolaurate	1.0	24	10	24	Flounder	Wildish 1974
Coal liquefied WSF[a] (total phenols)	1.54	48	~6.0	96	Fathead minnow	Gray 1990
Total residual chlorine	0.01		0.99–2.87		Rainbow trout	Cherry and Cairns 1982
Chlorinated waste	0.20	48	0.73–1.09	48	White sucker	Cherry et al. 1979
Free residual chorines	0.036				Fathead minnow	Larrick et al. 1978
Fire-Trol GTS-R	0.65		6.5	96	Rainbow trout	Wells et al. 2004
Phos-Chek D75R	1.3		>142	96	Rainbow trout	Wells et al. 2004
LAS	0.01				Japanese killifish	Hidaka and Tatsukawa 1989
Fenitrothion	0.09		6.8[b]	48	Japanese killifish	Hidaka and Tatsukawa 1989
Xylene	0.2		10	96	Coho salmon	Maynard and Weber 1981
α-Chloroacetophenone	0.05		1.05	48	Green sunfish	Summerfelt and Lewis 1967

[a]WSF, water-soluble fraction.
[b]Tsuda et al. 1997.

Table 2. Attraction–preference responses in fish to contaminants.

Pollutant	Sublethal concentration ($mg \cdot L^{-1}$)	Test duration (h)	LC_{50} ($mg \cdot L^{-1}$)	LC_{50} test duration (h)	Fish species	Reference
Coal liquefied WSF[a] (total phenols)	~2.0	48	~3.0	48	Rainbow trout	Gray 1990
Domestic sewage waste	20:1				Steelhead salmon	Smith and Bailey 1990
Oil refinery waste	1000:1 dilution ratio				Steelhead–Chinook salmon	Smith and Bailey 1990
Xylene (avoided)	0.01 (0.1)	1	10	96	Rainbow trout	Folmar 1976

[a]WSF, water-soluble fraction.

species of fish. Drummond and Carlson (1977) worked on test methods for the cough test and found that the cough frequencies were often predictive of more traditional long-term (chronic) effects at levels near the maximum allowable toxicant concentration (MATC) for many substances (Beitinger and McCauley 1990). Therefore, although the ecological significance of an increased cough rate in and of itself is not readily apparent, the thresholds discovered through the cough rate test may have some use in identifying concentrations of chronic concern even within the context of an acute test exposure.

Though chemoreception is often linked to avoidance and preference behaviour in fish, it also plays a role in other behaviours. For example, altered feeding behaviour may be directly linked to damage to particular sensory organs as a result of chemical exposures (e.g., smell). Sprague (1971) noted instances in which exposures to contaminants damaged taste buds, making fish unable to find food. Similarly, chemical exposures have been found to reduce the "palatability" of food, causing fish to spit out food (Sprague 1971; Sutterlin 1974). In both cases, these effects would be expected to impact negatively on survival. Contaminants can also impact the physical and decision-making abilities of organisms needed to locate, capture, and consume their food (Grue et al. 2002). Chemoreception plays a key role in fish detecting and reacting to chemical alarm cues and signals from predators and is thus vital to the survival of fish within their environment (Friesen and Chivers 2006). Contaminants can mask and obfuscate these signals in the environment, which may have ecologically significant consequences for affected organisms (Sutterlin 1974; Grue et al. 2002).

Although all avoidance–attraction, cough rate, predator–prey relationships, feeding, and appetite increases–decreases represent potential endpoints for measuring effects from sublethal aquatic exposures, not all chemicals cause the same set of behavioural responses and (or) intensity of response during sublethal exposures. Understanding the relationship between how a contaminant exerts its toxicity and the organism's response to the contaminant is considered one of the key elements in the prediction of behavioural effects. For example, a reduction in acetylcholinesterase (AChe inhibitors) is often associated with exposure to substances with reactive (or more specific) modes of action (MOA), e.g., the organophosphate insecticides, and may be a useful physiologic measure indicating potential for sublethal effects (Sprague 1971). The inhibition of AChe has implications for fish behaviour, as a reduction in this enzyme results in a buildup of the neurotransmitter acetylcholine (Heath 1995). Scott and Sloman (2004) cite a number of studies in which brain cholinesterase activity is linked to various behavioural changes such as changes in swimming and feeding behaviour.

Table 3. Sublethal effects on swimming behaviour caused by contaminants.

Pollutant	Sublethal endpoint	Sublethal concentration ($mg \cdot L^{-1}$)	Test duration (h)	LC_{50} ($mg \cdot L^{-1}$)	LC_{50} test duration (h)	Fish species	Reference
Carbaryl	Swimming capacity	1.0	96	1.95	96	Rainbow trout	Johnson and Finley 1980
Chlordane	Swimming activity	0.002	96	0.042	96	Rainbow trout	Little et al. 1990
Chlorpyrifos	Hypoactive	0.2	24	0.25	48	Japanese killifish	Rice et al. 1997
Diquat	Rheotaxis	0.5	24	90	24	Rainbow trout	Dodson and Mayfield 1979
LAS	Locomotor behaviour	0.03	24	>1		Zebra fish	Grillitsch et al. 1999
MS-222	Swimming (stimulatory)	60	2			Mummichog	Kane et al. 2004
Parathion	Swimming activity	0.01	96	3.7	96	Rainbow trout	Little et al. 1990
Permethrin	Hyper- then hypo-activity	0.010	24	0.011	48	Japanese killifish	Rice et al. 1997
Simazine	Rheotaxis	1.0	24	200	24	Rainbow trout	Dodson and Mayfield 1979
2,4-D	Swimming capacity	0.5–50	96	100	96	Rainbow trout	Little et al. 1990

Table 4. Other behavioural modifications caused by contaminants.

Pollutant	Sublethal endpoint	Sublethal concentration ($mg \cdot L^{-1}$)	Test duration (h)	LC_{50} ($mg \cdot L^{-1}$)	LC_{50} test duration (h)	Fish species	Reference
Carbofuran	Sheltering – burst swimming, agonistic behaviour	0.005–0.05	48	<0.5	96	Goldfish	Bretaud et al. 2002
Esfenvalerate	Social behaviour — agonistic behaviour	0.0001	44			Bluegill sunfish	Little et al. 1993
Lindane	Cough rate (gill purge)	0.006		0.009 MATC		Brook trout	Drummond and Carlson 1977
Malathion	Cough rate (gill purge)	0.007		<0.016 MATC		Brook trout	Drummond and Carlson 1977
Profenofos	Gill structure – locomotion effects	0.13	20 days	0.64	96	Mosquito fish	Venkateswara Rao et al. 2006
Sumithion	Feeding frequency	0.1	2	1.3	96	Coho salmon	Bull and McInerney 1974
DEF[a]	Strike frequency – prey capture	0.005	96	0.66	96	Rainbow trout	Little et al. 1990
Fluorene	Strike frequency – prey capture	0.06		0.91		Bluegill sunfish	Sandheinrich and Atchison 1990
Microcystin-LR	Temporal swimming patterns	0.005				Sunbleak	Baganz et al. 2005
Shale oil	Prey success	0.9	96	2.3	96	Fathead minnow	Woodward et al. 1987
No. 2 fuel oil	Feeding behaviour	0.019 ($ml \cdot L^{-1}$)				Catfish	Sandheinrich and Atchison 1990
Octylphenol	Courtship success	0.025	6 months			Japanese killifish	Gray et al. 1999
PCP[b]	Survival from predation	0.0002	96	0.052	96	Rainbow trout	Little et al. 1990

[a]DEF, organophosphorus defoliant *S,S,S*-tributyl-phosphorotrithioate.

[b]PCP, pentachlorophenol.

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The role of toxic mode of action (MOA) in behavioural effects assessment

How a chemical exerts its toxicity can be defined as its toxic mode of action (MOA). Sprague (1971) stressed the importance of MOA in understanding sublethal effects. Drummond et al. (1986) carried out important research into toxic MOA for fish, in which categories of behavioural effects were observed and associated with 139 chemicals. The work by Drummond et al. (1986) ranked indicators of stress to fathead minnows and provided valuable insight into which behaviours may provide the most sensitive measure of sublethal effects. The major thrust of this work was to determine if chemicals could be classified by their toxic MOA using behavioural and morphological indices. Chemicals that caused all fish to respond in a similar manner (i.e., behavioural response) were classified into particular groups based on a common toxic MOA. These were subsequently associated with the "most typical pattern of response" for that particular MOA. For example, substances acting by a narcotic MOA (i.e., narcosis), often referred to as baseline toxicity, were observed to cause fish to be hypoactive when spontaneous swimming behaviour was measured, whereas substances acting by an uncoupler of oxidative phosphorylation MOA caused a hyperactive response in the fish tested (Drummond et al. 1986). It was found by Drummond et al. (1986) that 84% of the 139 compounds tested ultimately resulted in a loss of equilibrium as an observable effect, regardless of MOA.

Drummond et al. (1986) evaluated 40 different signs of stress for their usefulness in classifying chemicals. Drummond and Russom (1990) built upon this work by examining the utility of categorizing chemicals based on three MOA-related response syndromes: hyperactivity, hypoactivity, and physical deformity syndrome. These so-called behavioural toxicity syndromes, which have also been referred to as fish acute toxicity syndromes (FATS) by McKim et al. (1987), allow for general relationships between behavioural toxicity syndromes and MOA. For example, a hypoactivity syndrome represents a pattern of response anticipated from chemicals acting via a narcotic MOA (Drummond and Russom 1990). McKim et al. (1987) developed FATS for five different categories of toxicants, which overlap with the MOAs identified by others in the field. McKim et al. (1987) investigated the use of FATS primarily for the purposes of quantitative structure activity relationship (QSAR) development, based on the premise that various structural features are related to particular MOAs and toxicity syndromes and, therefore, may prove amenable to modeling. Indeed, the work by Drummond et al. (1986), McKim et al. (1987), and Drummond and Russom (1990), among others, led to the development of a system to identify MOA based on chemical structure by Russom et al. (1997). A computer-based expert system (ASTER) was developed associating chemical structures with likely MOA. Also, in some cases, QSARs were developed in ASTER to estimate toxicity based on MOA in fathead minnow (Russom et al. 1997). Given the number of chemicals that may be subject to ERA internationally, it would seem worthwhile to give further consideration of toxic MOAs and their relationships with specific behavioural endpoints (or groups thereof). This could ultimately lead to consideration of behavioural effects within ERAs in a consistent and systematic fashion.

Current potential for behavioural toxicity information in ERAs

Behavioural and sublethal toxicity testing has progressed considerably in recent years, with more and more complex endpoints being examined in increasingly complex tests (e.g., Baganz et al. 2005; Kane et al. 2005). The role that computers play in this evolution in behavioural toxicity cannot be overlooked and bodes well for the future prospects of incorporating and interpreting these tests in a routine, consistent, and reliable manner. The acceptability of behavioural tests for hazard evaluation has increased along with increases in the technological capacity to measure and consistently quantify deviations in observed behaviour (Kondaiah and Murty 1994; Kane et al. 2004). Behavioural tests are now providing more defined and interpretable endpoints that meet the needs of at least some regulatory agencies in damage assessments and product registration and in the development of water quality criteria (Little and Brewer 2001). However, more work is still required to improve confidence in the reproducibility of results from these tests within the regulatory ERA community.

The standardization of behavioural toxicity assays also has been slow to progress. Recently, the American Society of Testing and Materials (ASTM) has made efforts in this direction and has developed a number of test protocols for behavioural testing in fish (Little 2002; American Society for Testing and Materials (ASTM) 2007). One of the limitations of behavioural research is that field validation is very difficult, which may be a hindrance to the widespread acceptance of this type of data (Little 2002). That being said, enough field validation has been conducted on avoidance that this endpoint is accepted as a useful endpoint within a number of pieces of legislation (Lipton et al. 1997; Little 2002). Over time, the continued advances in technology will likely allow for more opportunities for field validation (beyond avoidance) for a larger variety of behavioural endpoints (Little 2002; Gerhardt 2007). Examples of technologies that currently exist and that can be used for field validation of behavioural toxicity include telemetry, ultrasound, and infrared light beams, as well as the implementation of "on-line" biomonitors in various rivers around the globe (e.g., large rivers in Europe) (Gerhardt 2007). All of these advancements in technology and improvements in techniques, methodologies, and validation will not only help regulators gain confidence in this information, but also enable better understanding of how to interpret results and ultimately aid in its adoption within ERA frameworks

Current use of behavioural effects within regulatory frameworks

Although the uptake and use of behavioural toxicity endpoints in risk assessments and environmental protection initiatives by regulatory agencies have been slow, there are signs in recent years that this is starting to change (Steele et al. 1996; Little 2002). In particular, it has been recognized by scientists and regulators that changes in animal behaviour are among the most sensitive indicators of environmental disturbance (Gerhardt 2007). As a result, a num-

ber of pieces of legislation, particularly in the US, have explicitly referred to behavioural endpoints (most notably avoidance) as a substantive measure of environmental "injury" or damage (Steele et al. 1996; Little 2002).

In a review of the application of behavioural data in US environmental regulation, Little (2002) noted that many of the major pieces of legislation dealing with chemicals and the environment (e.g., Toxic Substances Control Act (TSCA)) do not explicitly make allowances for behavioural data. However, other legislation such as the Clean Water Act (CWA) and Oil Pollution Act (OPA) have allowed for the use of behavioural data as part of a weight-of-evidence approach. Notably, the CWA allows for the direct use of behavioural data such as cough responses from fish exposed to effluent as part of their discharge permitting process. The explicit mention of behavioural endpoints in fish (e.g., avoidance, signs of clinical toxicity, and AChe inhibition) as part of the Natural Resource Damage Assessments (NRDA) is often cited as evidence of the use of behavioural data within environmental regulation (Steele et al. 1996; Lipton et al. 1997; Little 2002).

Prospects for determining the ecological significance of effluent plumes in ERAs

The area impacted by lethal effects, or mortality, is usually confined to the vicinity of the source of pollution (i.e., the discharge point). From this point, source dispersion and dilution begins, with rates and downstream concentrations dependant on the nature of the contaminant and the physical and chemical characteristics of the receiving watercourse. The extent that regulatory authorities take such dispersion into account in the development of mixing-zone policy and regulation varies considerably. Though spatial criteria are often used to define mixing zones, a number of jurisdictions also incorporate biological considerations, some of which may be related to behavioural toxicity.

The biological considerations defined for mixing zones in jurisdictions such as Canada and the US usually relate to measures of acute lethality and (or) chronic endpoints observed in "classical" or standardized toxicity testing (e.g., prevention of the releases of acutely toxic concentrations of pollutants). However, this is not always the case, and behavioural endpoints such as maintenance and protection of safe passageway, migration routes, spawning areas, and biological communities may also be considered (US EPA 1988; US EPA 1994; CCME 2005). Consideration of "safe" passageway and migration routes for fish would seem to be (and is in some cases) an excellent means by which to incorporate avoidance behaviour information, particularly with respect to organisms that rely on chemoreception as part of their life cycles (e.g., salmon). Similarly, consideration of critical habitats and prevention of community–ecosystem interference could be understood to be based, at least in part, on behavioural considerations. A potential new use of behavioural toxicity information could be the incorporation into mixing-zone policy of criteria based on acute (short-term exposure) behavioural endpoints (e.g., avoidance). This would represent a significant improvement in the protection afforded to the biotic community by these policies.

Conclusions

The use of data on behavioural toxicity in ERAs could provide a great deal of value added to the assessment process, allowing for the consideration of more ecologically significant and protective hazard and exposure scenarios. One of the major advantages of behavioural effects is that they are more sensitive indicators of the potential for impacts on survival in the field than are measures of lethality. Currently, within ERAs carried out by most jurisdictions, the focus has been on traditional effect endpoints such as lethality. Lethality, however, represents the ultimate stress test and thus is not necessarily protective of the natural environment and the biota within it (Beitinger and McCauley 1990). Acute toxicity has been found not to be the best predictor of survival and effects in organisms (Little and Brewer 2001).

Potential applications are evident when examining the behavioural effects of contaminants that induce avoidance and changes in swimming behaviour in fish. These may occur, for example, at concentrations that are 1–2 orders of magnitude below those associated with acute toxicity of a substance; effects observed at 0.1%–5% of the acute LC_{50} values are not uncommon (Beitinger 1990; Little and Finger 1990). The implications of effects at those levels go beyond the behaviour of individual organisms, extending to population-level effects and even to effects on entire habitats. Appropriate habitat is critical to the survival of individuals and their offspring and, indeed, of populations (Little and Brewer 2001).

The use of sublethal behavioural effects when determining critical effects concentrations in ERAs may require some additional considerations, for example, when the behavioural effects reported have been observed under short-term (acute) exposure conditions. In this situation, it is not clear whether additional extrapolation factors would be warranted to account for uncertainties associated with longer-term (chronic) exposures. That being said, behavioural effects may prove to be most useful when defining predicted no-effect concentrations (PNECs) for use in quantitative risk calculations as a more sensitive acute, or even chronic, threshold value for the purposes of interpreting the ecological significance of effluent plumes and (or) instream concentrations.

There is no doubt that there is potential for greater use of information on behavioural toxicity within ERAs conducted by regulatory agencies. To a certain extent, this type of information is already being used by regulators, but mostly on an ad hoc basis. However, the lack of standardized protocols continues to be an issue that hinders the acceptance of this information by the regulatory community. In particular, further work building on that of McKim et al. (1987), Drummond and Russom (1990), and Russom et al. (1997), for example, would also be desirable. The use of QSAR and other computational models aimed at linking MOA and behavioural toxicity in a more systematic and consistent manner across a wide variety of organic chemicals would be helpful. However, what is perhaps most critical at this point is for risk assessors to begin to use, in regulatory ERAs, the information that currently exists. This will help to build the capacity within regulatory agencies to use and interpret this information when assessing ecological risks.

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PERSPECTIVE / PERSPECTIVE

Limnology in northeastern Ontario: from acidification to multiple stressors[1]

W. (Bill) Keller

Abstract: Thousands of lakes around Sudbury, in northeastern Ontario, Canada, were badly damaged by acid deposition and many were also metal-contaminated. Large reductions in atmospheric sulphur and metal emissions have led to widespread chemical improvements in these lakes, and recovery has been documented for various biota. These findings were very important in establishing the necessity and value of sulphur emission controls during the international debates about the effects of acid deposition and the need for cleaner air. Studies of northeastern Ontario lakes are continuing to advance our understanding of chemical and biological recovery processes; however, that knowledge is still incomplete. It has become apparent that the recovery of lakes from acidification is closely linked with the responses to, and interactions with, other large-scale environmental stressors like climate change and calcium declines. Developing a better understanding of lake recovery processes and their future outcomes within such a multiple stressor context will be difficult. It will demand the merging of various approaches, including monitoring, experimentation, paleolimnology, and modelling, and will require effective collaboration among different research and monitoring sites and various agencies and institutions engaged in environmental science.

Résumé : Des milliers de lacs des environs de Sudbury, dans le nord-est de l'Ontario, Canada, ont été lourdement endommagés par les dépôts acides et plusieurs ont été aussi contaminés par des métaux. Les réductions importantes des émissions atmosphériques de soufre et de métaux ont conduit à une amélioration générale de la chimie de ces lacs et on a démontré le rétablissement de divers organismes. Ces résultats ont été de grande importance pour établir la nécessité et l'importance du contrôle des émissions de soufre lors des débats internationaux sur les effets des dépôts acides et la nécessité d'air plus pur. Les études faites sur les lacs du nord-est de l'Ontario continuent à faire progresser notre compréhension des processus de rétablissement chimique et biologique; ces connaissances demeurent cependant encore incomplètes. Il est devenu évident que la récupération des lacs de l'acidification est reliée étroitement à leurs réactions aux autres sources de stress à grande échelle dans l'environnement, tels que le changement climatique et le déclin du calcium, ainsi qu'à leurs interactions avec ces sources. Il sera difficile d'obtenir une meilleure compréhension des processus de récupération des lacs et de leur destin futur dans un tel contexte de sources multiples de stress. Cela nécessitera la conjonction de plusieurs approches dont la surveillance continue, l'expérimentation, la paléolimnologie et la modélisation; il faudra aussi une coordination effective entre les divers sites de recherche et de surveillance, ainsi qu'une collaboration des différentes agences et institutions impliquées dans les sciences de l'environnement.

[Traduit par la Rédaction]

Introduction

During the 1980s, the acidification of lakes by acid deposition emerged as a major international concern (e.g., Cowling 1982), although acid rain effects on lakes had been identified much earlier (e.g., Oden 1968). In fact, the 1980s are sometimes referred to as the acid rain decade, since that was the period when scientific, public, and political recognition of the issue came to the forefront, and large efforts were mounted to begin to understand and address the problem.

Large-scale lake liming programs were initiated to combat acidification in some areas, notably in Scandinavia (Olem et al. 1991; Henrikson and Brodin 1995). In North America, lake liming was never adopted as a widespread management tool, although experiments were conducted to examine the feasibility of liming and determine the responses of aquatic biota to the neutralization of lake acidity (Porcella 1989; Keller et al. 1990*a*). Because of the large numbers and general inaccessibility of acidified lakes in North America (an

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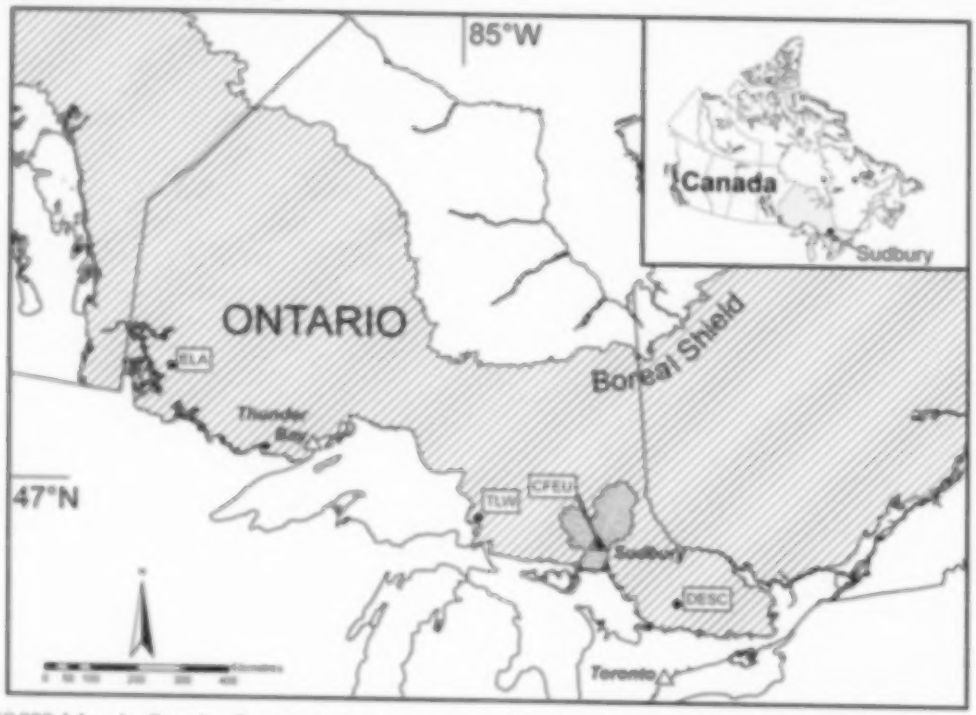
[1]This Perspective is based on the author's F.H. Rigler Lecture delivered at the annual meeting of the Society of Canadian Limnologists in Halifax, Nova Scotia, January 2008.

Fig. 1. The location of Sudbury in the Boreal Shield Ecozone (diagonal shading), the historical lake damage zone around Sudbury (stippling), and locations of long-term aquatic research and monitoring sites in Ontario: DESC (Dorset Environmental Science Centre); CFEU (Cooperative Freshwater Ecology Unit; www.livingwithlakes.ca); TLW (Turkey Lakes Watershed; www.tlws.ca); ELA (Experimental Lakes Area; www.umanitoba.ca/institutes/fisheries/).



estimated 19 000 lakes in Ontario, Canada, alone; Neary et al. 1990) extensive liming was not considered to be logistically or economically feasible. Also, since liming only treats the symptoms and not the causes of the acid deposition problem, control programs to reduce acid emissions were given priority by government agencies in Canada and the USA.

Efforts to achieve sulphur emission controls were eventually successful. Under various agreements, both Canada and the USA substantially reduced sulphur dioxide emissions. Total North American emissions are now about 40% less than in 1980 (Jeffries et al. 2003). However, these large-scale emission reduction programs were in reality still just massive experiments, since the ultimate outcome for aquatic ecosystem recovery was far from certain.

The Sudbury area of northeastern Ontario, Canada, provides one of the best examples in the world of the ecological effects of sulphur emissions and their control and is the case study I focus on here. Sudbury (Fig. 1) is located roughly at the centre of the Boreal Shield, Canada's largest ecozone, which contains 22% of Canada's freshwater surface area (Urquizo et al. 2000). Typical of the Boreal Shield, lakes in northeastern Ontario are generally low in ionic strength, nutrient poor, and very sensitive to anthropogenic influences, including acid deposition. Over a century of sulphur and metal particulate emissions from metal smelters led to widespread terrestrial and aquatic damage near Sudbury (Gunn 1995). It is estimated that over 7000 lakes in a 17 000 km^2 area around Sudbury were acidified to the point that biological damage occurred (Neary et al. 1990; Fig. 1). Many of the lakes closest to the Sudbury smelters were also highly metal-contaminated (Keller and Pitblado 1986). However, large-scale emission reduction programs were implemented at the Sudbury smelters in the 1970s and 1990s, achieving overall reductions of ~90% in sulphur (Fig. 2*a*) and metal emissions in comparison with the peak emission levels in the 1960s (Potvin and Negusanti 1995; Keller et al. 2007).

Lake recovery

Have sulphur emission reduction programs worked? The answer is clearly yes, or perhaps more accurately, yes, they *are* working. The acid rain story is still far from over in Canada and in other areas of the world affected by excessive acid deposition. However, very encouraging evidence of chemical and biological recovery of lakes following reductions in acid deposition is emerging, particularly in the Sudbury area, where emission controls were implemented earlier than in most other acid-affected regions and where emission reductions were very large (Keller et al. 1999*a*).

In northeastern Ontario, changes in lake chemistry, including increased pH and reduced sulphate and metal concentrations, followed the major reductions in emissions from the Sudbury area smelters in the 1970s and 1990s (Keller and Pitblado 1986; Keller et al. 1992*a*; Keller et al. 2007). While many of the thousands of historically damaged lakes are still acidic, the pH of some formerly acidified lakes has increased to >6.0 (Keller et al. 2007), a widely accepted threshold for effects on many acid-sensitive aquatic

species (Keller et al. 1990*b*; Havens et al. 1993; Holt and Yan 2003). Changes in the sulphate concentrations and pH of Clearwater Lake near Sudbury over the last several decades provide a dramatic example of this chemical recovery from acidification (Fig. 2*b*), which has been accompanied by increases in dissolved organic carbon (DOC) concentrations and decreases in calcium concentrations (Fig. 2*c*). The metal concentrations in many lakes have declined greatly with time, including metals related to acid leaching of watersheds (e.g., aluminum, manganese) and smelter-related metals (e.g., copper, nickel). However, despite dramatic declines, concentrations of some smelter-related metals, including copper and nickel, remain elevated in lakes close (within ~30 km) to Sudbury (Keller et al. 1999*b*).

Following water quality improvements, evidence of biological recovery has emerged for many groups of aquatic organisms, including phytoplankton (Nicholls et al. 1992; Graham et al. 2007; Winter et al. 2008), zooplankton (Keller and Yan 1991; Locke et al. 1994; Keller et al. 2002), benthic invertebrates (Griffiths and Keller 1992; Snucins 2003), and fish (Gunn and Keller 1990) in Sudbury area lakes. However, it has also become apparent that biological recovery can be a very complex process that we cannot yet predict with confidence.

Improvements in biological communities may not simply follow improvements in chemical habitat quality. Altered food webs resulting from previous chemical damage can continue to control community structure even after suitable chemistry is restored. In lakes where planktivorous fish were eliminated by acidification, abundant populations of invertebrate predators usually controlled by fish predation, like *Chaoborus*, may control zooplankton communities (Yan et al. 1991; Keller et al. 2002). In cases where some fish have persisted or are becoming reestablished in recovering lakes, unusually simple fish communities such as abundant populations of the acid-tolerant yellow perch (*Perca flavescens*) may also retard zooplankton recovery (Yan et al. 2004). Such "biological resistance" (Keller and Yan 1998) can be a major factor affecting recovery patterns in lakes after the removal of chemical stress. As well, many other poorly understood factors can be expected to influence biological recovery processes, including various physical and biological constraints on the ability of different organisms to disperse and effectively colonize lakes (Keller and Yan 1998; Yan et al. 2003). Examples of complicating factors include possible suppression of colonists by established acid-tolerant zooplankton (Binks et al. 2005) and within-species adaptations in acid tolerance (Derry and Arnott 2007). The relative influence of different factors may be very lake specific.

Various conceptual models have been proposed as frameworks for the study of lake recovery processes (Keller and Yan 1998; Yan et al. 2003; Keller et al. 2007). However, we are far from being to able to realistically model biological recovery in a quantitative sense. Attempts to model biological recovery to date have had to rely only on direct relationships observed between species distributions and lake chemistry (e.g., Doka et al. 2003) without the ability to incorporate the many biological and physical factors affecting recovery processes. Our incomplete understanding of the roles of various physical and biological factors in affecting recovery processes in lakes of different types will need to be much improved to enhance predictive abilities for future biological recovery.

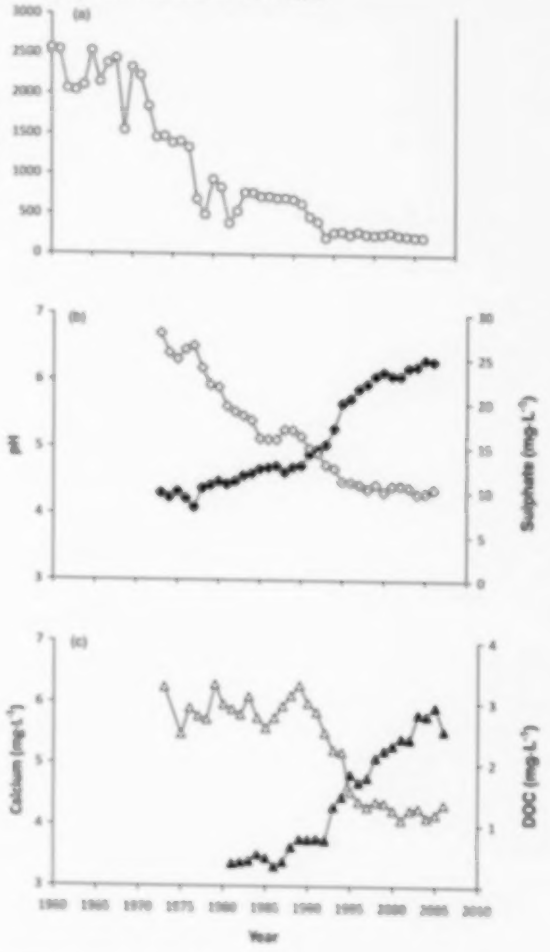


Fig. 2. (*a*) Annual sulphur dioxide emissions (kilotonnes (kt)) from Sudbury area smelters, (*b*) average annual pH (◆) and sulphate concentrations (◊), and (*c*) calcium (Δ) and dissolved organic carbon (DOC, ▲) concentrations for Clearwater Lake. Clearwater Lake, 13 km from Sudbury, has the longest continuous monitoring record of any acidified lake in the world.

Surprises

Many of the chemical changes in Sudbury area lakes that followed emission reductions, such as increased pH and reduced sulphate concentrations, were anticipated. Other important changes came as surprises, revealed by the monitoring programs implemented to study acidification.

Long-term monitoring data for northeastern Ontario lakes clearly demonstrated that the declines in lake water sulphate due to reductions in sulphur deposition were not simply balanced by declines in lake acidity, but also by substantial declines in base cation concentrations. Declines in calcium concentrations (Keller et al. 2001*a*) were of particular con-

cern because of the implications for biota. Many important organisms have relatively high calcium requirements that can affect their distributions (Tessier and Horwitz 1990; Hessen et al. 1995), and calcium modifies the effects on biota of many other stressors, such as acidity and metals (Brown 1983), ultraviolet (UV) radiation (Hessen and Alstad Rukke 2000), and temperature (Ashforth and Yan 2008). Recent investigations have shown that calcium declines throughout much of Ontario are in fact a major threat to the biota of soft-water Precambrian Shield lakes, with effects already evident on *Daphnia*, a calcium-rich taxon very important in aquatic food webs (Jeziorski et al. 2008). Declines in base cations, including calcium, are widespread in regions recovering from acidification (Stoddard et al. 1999). These declines likely are a result of reduced current acid leaching rates and depletion of exchangeable base cation pools in lake watersheds by many years of elevated cation export. However, other factors, including base cation removal through forest harvesting and regrowth, may be very important regionally (Watmough and Dillon 2003)

Monitoring has also revealed other important surprises. Swan Lake, near Sudbury, like many other area lakes, was increasing in pH during the early to mid-1980s, when abruptly the pH declined from ~5.7 to ~4.5 in 1988. Acid deposition did not change during that time; the change that caused the re-acidification of Swan Lake was a change in the weather. During a prolonged drought (1986–1987), watershed soils and littoral sediments dried, and stored sulphur and metals were oxidized and later mobilized into the lake water when wet conditions resumed (Keller et al. 1992*a*). The re-acidification profoundly changed the nature of the lake in many ways, including increasing metal concentrations, transparency, UV-B penetration, and bottom temperatures (Yan et al. 1996*a*), with dramatic effects on phytoplankton and zooplankton communities (Arnott et al. 2001). Two decades ago, Swan Lake provided a very strong signal of some of the dramatic changes that might result from climate change in the future and helped reveal the potential importance of interactions between different large-scale stressors, including acidification, climate change, and ozone depletion.

Clearly, the usefulness of long-term studies extends far beyond the direct assessment of changes related to specific environmental control measures. The surprises revealed by monitoring can be crucial to identifying new threats that need to be addressed.

Land–water linkages

The above examples (widespread calcium decline and the re-acidification of Swan Lake) also illustrate very well something that we all know; lakes are intimately linked to their watersheds. Watershed processes, not just lake processes, will strongly affect lakes and lake recovery. If we remove important ecosystem components like calcium from the landscape through anthropogenic activities, we will have to wait a long time for weathering to replace them. Contaminants like sulphur and metals stored in saturated watershed soils, lake sediments, and wetlands are not gone, but can be mobilized, particularly through the effects of droughts, as has been observed near Sudbury (Keller et al. 1992*a*) and in south-central Ontario (Dillon et al. 1997; Adkinson et al. 2008). When we store contaminants on the landscape, we create a legacy that will persist for an unknown time period. It has been estimated that the effects of metals deposited in the watersheds of some of the most severely affected Sudbury lakes may continue for centuries (Nriagu et al. 1998).

Furthermore, watersheds around Sudbury that were denuded by very harsh historical logging and smelting processes do not provide the woody debris for nearshore cover or the typical amounts and types of organic material (Rasmussen et al. 2008) important to the functioning of littoral lake habitats (Roth et al. 2007; Helmus and Sass 2008). Forest regrowth on formerly denuded watersheds around Sudbury has already reduced wind speeds, which in turn has affected lake thermal structure by reducing wind-induced mixing (Tanentzap et al. 2008); however, reestablishment of healthy forests in such badly damaged landscapes is a very slow process that may sometimes require human intervention (Courtin 1995). As well, many of the major impacts of climate change on small Boreal Shield lakes will likely be through effects on watershed processes such as organic matter production and decomposition, with resultant effects on lake clarity and thermal structure (Keller et al. 2008) underscoring the need for a better understanding of key land–water linkages.

Additional process-oriented research is needed on the important linkages between lakes and their watersheds. Effective management of recovering lakes near Sudbury and elsewhere clearly needs to consider watershed management strategies, not simply lake management techniques.

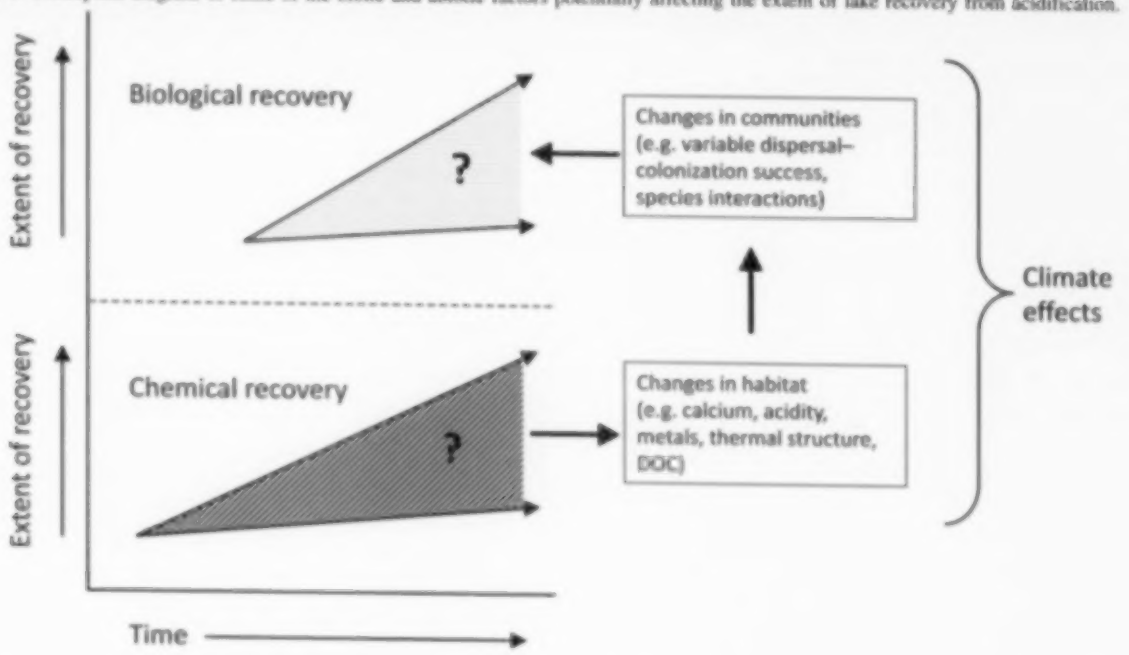
Multiple stressors, multiple approaches

We cannot address major environmental issues like acidification, or understand processes like recovery from acidification, in isolation from other large-scale stressors affecting aquatic ecosystems. Multiple interacting stressors are the reality in many of today's aquatic environments (Sala et al. 2000). Stressors such as acidification, base cation depletion, and invasive species — all under the overarching influence of a changing climate (Fig. 3) — will interact and affect lakes in ways we cannot yet predict with confidence (Schindler 2001; Keller 2007; Yan et al. 2008).

Understanding the effects of multiple stressors will be a complex undertaking and will demand assessment approaches that will also be complex and multidisciplinary. Using studies on lakes in northeastern Ontario as examples, it can be seen that much of the success of the acidification science programs that ultimately led to the widespread implementation of sulphur emission controls was due to the effective merging of results from different scientific approaches. These included temporal and spatial surveys, paleolimnological reconstructions, laboratory and field toxicity tests, and lake-scale experimental liming studies.

Keller et al. (1990*b*) combined results from large spatial surveys, laboratory toxicity studies, and a whole-lake neutralization experiment to demonstrate that widespread losses of the important, widely distributed crustacean zooplankter, *Daphnia mendotae*, had resulted from acidification and that these losses had occurred in lakes below a pH threshold of ~6.0. Such studies were instrumental in making the case for

Fig. 3. Conceptual diagram of some of the biotic and abiotic factors potentially affecting the extent of lake recovery from acidification.



the occurrence of extensive biological damage even at what were then considered comparatively moderate levels of lake acidity. This was a case that needed to be made internationally, not just to scientists but also to policy makers, to demonstrate the critical need for sulphur emission controls.

Paleolimnological reconstructions of lake acidity histories in many northeastern Ontario lakes (Dixit et al. 1992; Vinebrooke et al. 2002) revealed widespread acidification and showed that natural acidity could not account for the extensive occurrence of acid lakes observed in contemporary large-scale field surveys (Keller and Pitblado 1986). This effectively countered early arguments that lake acidification was predominantly a natural process (Krug et al. 1985). Temporal comparisons of monitoring data (Keller and Pitblado 1986; Keller et al. 1992*a*) demonstrated that chemical recovery would follow reductions in acid deposition, and paleoecological reconstructions (Dixit et al. 1989), observations from naturally recovering lakes (Gunn and Keller 1990; Keller and Yan 1991), and liming experiments (Keller et al. 1992*b*; Yan et al. 1996*b*) showed that biological recovery could be expected to follow chemical improvements in aquatic habitats. In combination, findings from these studies provided a compelling case for the ecological benefits of sulphur emission controls.

The examples given above show how findings from multiple approaches were combined to make a strong scientific case for acid rain damage and demonstrate the clear need for, and benefits of, sulphur emission controls. Knowledge generated through a variety of scientific strategies came together to clearly show that widespread lake acidification was directly due to excessive acid deposition, that lake acidification had caused extensive damage to lake communities, and that removal of acid stress would stimulate the chemical and biological recovery of aquatic ecosystems. The successful combination and dissemination of knowledge from investigations using different approaches and conducted at a variety of scales was essential in helping raise the scientific and public concern and ultimately stimulate the political will to address the acidification issue, although there were many concerns about the difficulty of effectively linking acid rain science and policy (e.g., Alm 2003). This scientific knowledge, based on many publications in the primary literature, was summarized in a number of comprehensive synthesis reports (e.g., RMCC 1990; NAPAP 1991) for communication to stakeholders and decision makers.

Multiple supporting lines of evidence make a very strong case, an important lesson learned from the acid rain issue, and something to remember as we confront other large-scale threats like climate change in a multiple-stressor world. We need to take on these environmental challenges together, not from individual research silos.

Reference conditions

Assessment of lake damage or recovery depends on having a measure of "normal" for comparison. Normal, however, may be difficult to define. Since predisturbance conditions are often not precisely known, normal must generally be defined by what is considered typical for regional lakes or lake types that are undisturbed or minimally disturbed. Lakes of interest for damage and recovery assessments are compared either visually or statistically with temporal (monitored through time) or spatial (synoptic surveys) reference lakes or better yet with both types of reference lakes (e.g., Keller et al. 1992*b*; Yan et al. 1996*b*). The dilemma of course lies in the availability and selection of

appropriate reference lakes, similar in attributes to the test lake but not affected by the particular stressor(s) under investigation. Of necessity, groups of reference lakes are often used to define the normal range of variability (Kilgour et al. 1998) for minimally disturbed systems against which test systems can be compared. While this approach has proved to be very useful for recovery assessments, the true appropriateness of statistical definitions of normal for comparisons with specific test lakes is difficult to judge.

Paleolimnological reconstructions can tell us much about the actual prestress conditions for many chemical and biological attributes of lakes (Smol 2008). However, historical conditions in a given lake may not necessarily represent the appropriate reference condition for contemporary assessments. It was recognized decades ago that given changes to other parts of disturbed ecosystems, a return to predisturbance communities within a reasonable time frame may not always be possible even after a stress is removed (Cairns 1990).

Studies in Ontario over the last three decades have demonstrated broadscale temporal changes in many key attributes of lake habitats, including concentrations of calcium, DOC, and phosphorus (Jeziorski et al. 2008; Keller et al. 2008; Yan et al. 2008). Recovery targets determined from reference lakes that are affected by multiple large-scale regional or global influences will be moving targets, underscoring the need for maintaining up-to-date, appropriate, reference data sets as part of long-term lake monitoring programs, to allow accurate assessments of the effects of environmental stressors.

A better understanding of the specific linkages between important aquatic habitat attributes and aquatic communities under reference conditions and the development of improved methods for reference condition definition based on habitat characteristics (Bowman and Somers 2005; Linke et al. 2005; Whittier et al. 2007) would greatly help with the assessment of stressor effects and recovery from them. More standardized definitions of what we actually mean by reference conditions for particular situations would also aid in the effective application of reference condition approaches to aquatic assessments (Stoddard et al. 2006).

The value of monitoring

Monitoring programs have sometimes been unfairly criticized as being unnecessary and expensive. In reality, monitoring is essential, for among many other purposes, determining what has been accomplished through environmental management programs and what further actions are required. The costs of environmental monitoring programs in fact are usually a very small fraction, much less than 1%, of the implementation costs or expected benefits of pollution control programs (Lovett et al. 2007).

Wise lake management depends on understanding the aquatic resources we are trying to manage. Long-term monitoring is an essential element in developing and maintaining that knowledge. As outlined in the previous examples, long-term lake monitoring studies in northeastern Ontario have played an essential role in documenting the responses of acidified lakes to sulphur emission controls, findings that were important not just regionally, but nationally and internationally in the debates for cleaner air. These studies have also played an important role in identifying and raising awareness of new stressors like calcium depletion (Keller et al. 2001*a*) and drought effects (Yan et al. 1996*a*) that may fundamentally change the nature of many aquatic ecosystems in the future.

Lake monitoring studies in northeastern Ontario have, in their own right, made large contributions to understanding the effects of multiple stressors on Boreal Shield lakes. However, one of the greatest values of such regional studies comes when they are merged with the results of studies from other long-term research and monitoring sites to address environmental issues on much broader geographical scales: for example, the extent of calcium declines in Ontario (Jeziorski et al. 2008), chemical recovery from acidification in eastern Canada (Clair et al. 2007), future risk from UV-B penetration across Canada (Molot et al. 2004), or recent changes in DOC concentrations in the Northern Hemisphere (Monteith et al. 2007).

Each of the major long-term limnological research and monitoring sites in Ontario, for example (Fig. 1), including the lakes studied at the Dorset Environmental Science Centre (south-central Ontario), the Cooperative Freshwater Ecology Unit (northeastern Ontario), the Turkey Lakes Watershed (Algoma Region), and the Experimental Lakes Area (northwestern Ontario), captures a unique set of characteristics and differing ecological histories, reflecting different regions of the Boreal Shield. Collectively these sites span large gradients in various stressors such as acid deposition, climate, and extent of shoreline development (Keller et al. 2001*b*). Interagency cooperation in effect links these sites into a network, where through collaborations the ability of the whole to answer science questions becomes much greater than the sum of the parts. Such collaborations need to be encouraged and strengthened.

As already demonstrated, data from long-term monitoring sites are valuable for many purposes beyond simply allowing us to track how aquatic ecosystems have responded to stressors or the removal of stressors. Monitoring data form the baseline from which important ecological hypotheses can be constructed and from which realistic experiments can be designed to test them. For example, the previously mentioned drought-induced acidification event observed in the Swan Lake monitoring record stimulated in-lake experimentation investigating the role of drought and re-acidification on zooplankton emergence from sediments (Arnott and Yan 2002). Long-term monitoring data also allow determination of the direct and indirect relationships between important lake attributes and stressors, information that can help us use the past to forecast the future of our lakes through the construction of empirical and conceptual models.

Lakes, as low points in the local landscape, integrate the effects of changes in processes within their watersheds as well as changes in internal processes. Lakes and specific lake attributes can be extremely important indicators of environmental change, notably in a climate change context (Williamson et al. 2009), but also for changes related to many other environmental stressors. The usefulness of lakes and lake attributes for tracking and understanding environmental changes is, however, only as good as the quality and security of the monitoring programs that employ them. The

maintenance of well designed and carefully operated long-term aquatic research and monitoring studies needs to be an integral part of environmental protection and management systems.

What now?

A decade ago, four research questions were identified for recovering lakes in the Sudbury area (Keller et al. 1999*b*). If we revisit these questions with a view to what has changed, it is evident that while our knowledge has advanced over the last decade (e.g., Keller et al. 2007), much more needs to be learned, and these questions still remain valuable as general guides for future research.

What are the interactive effects of lake acidification and other large-scale stressors such as climate change and UV-B irradiance?

While our understanding of multiple stressor effects is improving, it is still very incomplete. The potentially very severe implications of new stressors such as calcium depletion and invasive species introductions are now recognized, but how these and other large-scale stressors like climate change will interact to structure future lake environments is still poorly understood (Christensen et al. 2006). We do know that the indirect effects of stressors and stressor interactions, manifested through altered species interactions, will likely play a major role in structuring aquatic communities in the future. To advance our scientific understanding of lake recovery against an increasingly complex background, future studies will need to be conducted within a multiple stressor, multidisciplinary framework.

What will be the legacy of atmospheric contaminants such as sulphur and metals stored in the environment?

We have known for many years that the large amounts of pollutants, such as sulphur, stored in wetlands, lake sediments, and saturated watershed soils, can be mobilized with severe consequences for aquatic ecosystems. However, we still do not know the magnitude of these contaminant pools, the realistic time frames over which periodic mobilization might continue, and the probable long-term effects on aquatic communities. Such legacy issues will of course be closely tied to the overarching effects of changes in climate, again arguing strongly for future study in a multiple stressor context. Our recognition of important legacy issues in the Sudbury area and elsewhere now also includes not just the storage of contaminants but the removal of important elements like calcium from watersheds and the continuing effects of severe historical watershed disturbances (forest damage, soil erosion) on the physical and chemical characteristics of some recovering lakes.

Which organisms are capable of unassisted recovery and which are not?

Over the last decade, our understanding of biological recovery processes has advanced substantially, but we are still not in a position to clearly address this question for more than a few species. There are contrasting results showing widely varying patterns of recovery of aquatic communities in different studies. For example, crustacean zooplankton showed stronger evidence of recovery in species richness than in community composition in Whitepine Lake after pH 6.0 was reached (Keller et al. 2002), while in contrast, in a group of chemically recovered (pH > 6.0) Killarney area lakes, community composition but not species richness showed evidence of recovery (Holt and Yan 2003). Considering phytoplankton, while limited recovery has been observed in many Sudbury area lakes (Graham et al. 2007), complete community recovery appears to have occurred in Clearwater Lake (Winter et al. 2008), which is recovering from very severe acidification and metal contamination (Fig. 2*b*). Our understanding of the relative roles of different chemical, physical, and biological factors in controlling the recovery of lakes of different types is still very incomplete. In particular, the effects of biological interactions within the altered food webs in disturbed lakes and the effects of the various biological and physical factors controlling species dispersal and colonization success are very poorly known. Substantial additional research is required to develop more refined models to permit prediction of future biological recovery and identify additional management actions that may be needed to promote ecosystem recovery.

What are reasonable recovery targets?

There is recognition that the predisturbance condition may not necessarily be an appropriate target for aquatic ecosystem recovery. It is also apparent that the future reference conditions appropriate to gauge the recovery of acidified lakes may be a moving target because of changes in other large-scale stressors. This emphasizes the continuing need for developing and applying better methods for selecting and using aquatic reference data for assessments of both recovery and effects. It also argues strongly for the incorporation of reference data collection as an integral part of long-term monitoring programs designed to track the effects of various environmental stressors.

The future of recovering lakes in northeastern Ontario and in other regions of the world affected by excessive acid deposition is far from clear. Additional knowledge in the above research areas would contribute substantially to our ability to understand recovery processes and realistically forecast future lake recovery in a multiple stressor environment. The recovery story is becoming more complex as our understanding develops. Unravelling it further will demand effective collaboration between various agencies and institutions engaged in environmental science and management and will require the continuing synthesis of findings from various complementary scientific approaches.

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